UNIVERSIDADE DE LISBOA

Faculdade de Medicina Veterinária

PROSPECTIVE STUDY OF THE UTILITY OF IRIS AKI GRADING SYSTEM IN DETECTING RENAL INJURY IN PATIENTS PRESENTED WITH PROBABLE NEPHROTOXIN EXPOSURE AT A 1ST OPINION VETERINARY HOSPITAL

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DISSERTAÇÃO DE MESTRADO INTEGRADO EM MEDICINA VETERINÁRIA

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“To Know that we know what we know,
and to know that we do not know what we do not know,
that is true knowledge.”

Nicolaus Copernicus

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ABSTRACT

PROSPECTIVE STUDY OF THE UTILITY OF IRIS AKI GRADING SYSTEM IN DETECTING RENAL INJURY IN PATIENTS PRESENTED WITH PROBABLE NEPHROTOXIN EXPOSURE AT A 1ST OPINION VETERINARY HOSPITAL

Kidney diseases are common in dogs and cats and slight changes in kidney function do not result in organ failure but have an important role on the impact of the condition. Acute kidney injury (AKI) has been associated with high mortality and morbidity rates, and the early recognition of the disease has become necessary, to be able to use more effective therapeutic interventions, to recognise developing injury earlier and have more positive outcomes.

In 2013, the International Renal Interest Society (IRIS) adopted a grading system to categorize the severity of AKI, but this criteria has not yet been validated for use in general practice population. This study aimed to relate the detection of AKI using the IRIS criteria in small animals exposed to a nephrotoxin compared with the detection of AKI using the creatinine reference range alone.

The study population were client-owned dogs and cats presented at Village Vet Hampstead, a 1st opinion hospital practice (RCVS recognised), with history of exposure to a nephrotoxin. This included 11 feline patients (*n*=11) exposed to *Lilium* spp. and 7 canine patients (*n*=7) exposed to *Vitis* spp., which were later divided into two subgroups, depending on the development of AKI. In the feline sample, results demonstrated that both creatinine reference range and IRIS criteria identified AKI in the same feline patients (*n*=2), and when both methods were compared regarding sensitivity a *p* value of 1, showed no significantly difference between them regarding to sensitivity. In canine sample results showed that none of the dogs developed AKI. This was already expected since all patients were managed with fluids and monitoring, and incidence of AKI with treatment is only 8.5%. Therefore, less than 1 patient in every 10 are expected to have AKI, which corresponded to what was observed.

The study was underpowered, with a power calculation of 16%, which did not allow any statistically significant results. To improve the power calculation of this study, larger samples would be required.

This was a pilot stud and additional studies involving a larger sample size and funding are required to evaluate the sensitivity of IRIS AKI grading criteria when compared to the creatinine reference range alone.

Keywords: Acute Kidney Injury; Nephrotoxin; Lilies; Grapes.

RESUMO

ESTUDO PROSPETIVO DA UTILIDADE DO SISTEMA DE CLASSIFICAÇÃO DA IRIS NA DETEÇÃO DE LESÃO RENAL AGUDA EM PACIENTES QUE SE APRESENTAM COM PROVÁVEL EXPOSIÇÃO A UMA NEFROTOXINA NUM HOSPITAL VETERINÁRIO DE 1ª OPINIÃO

As doenças renais são comuns em cães e gatos, e mesmo pequenas alterações na função renal têm um papel importante no impacto da doença. A Insuficiência Renal Aguda (IRA) está associada a taxas de mortalidade e morbilidade elevadas, tornando-se necessário reconhecer precocemente a doença, para que possam ser realizadas terapêuticas eficazes, para que se reconheça o desenvolvimento de lesão mais cedo e para que os prognósticos sejam melhores.

Em 2013, a International Renal Interest Society (IRIS) adotou um sistema de classificação para categorizar a gravidade da IRA, mas este ainda não foi validado. O objetivo do presente estudo foi relacionar a deteção de IRA usando o critério da IRIS em comparação com o uso do intervalo de referência da creatinina, em pequenos animais expostos a uma nefrotoxina.

A população em estudo era composta por cães e gatos que se apresentaram no Village Vet Hampstead, um hospital de primeira opinião, com história de exposição a uma nefrotoxina. O estudo incluiu 11 felídeos (n=11) expostos a *Lilium* spp. e 7 canídeos (n=7) expostos a *Vitis* spp., os quais foram, posteriormente, divididos em dois subgrupos, dependendo do desenvolvimento de IRA. No grupo de felinos, os resultados demostraram que, tanto o intervalo de referência da creatinina como o critério da IRIS, identificaram IRA nos mesmos pacientes (n=2), e quando os dois métodos foram comparados relativamente à sua sensibilidade, um valor p de 1 mostrou que não haviam diferenças significativas entre a sensibilidade dos dois métodos. No grupo de canídeos, verificou se que nenhum dos pacientes desenvolveu IRA. A incidência de IRA com tratamento é de 8.5%, e todos os pacientes foram monitorizados e receberam fluidoterapia. Assim sendo, seria de esperar que menos de 1 paciente em cada 10 desenvolve se IRA, o que correspondeu ao observado neste estudo.

O poder do teste foi de 16%, valores tão baixos não permitem resultados estatisticamente significativos. Para melhorar o poder de teste, seriam necessárias amostras maiores.

Este estudo foi um estudo piloto, pelo que são necessários estudos adicionais, que envolvam amostras maiores e financiamento, para avaliar a sensibilidade do critério de IRA da IRIS quando comparado ao intervalo de referência da creatinina.

Palavras-chave: Insuficiência Renal Aguda; Nefrotoxina; Lírios; Uvas.

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LIST OF ABBREVIATIONS

ADMA – Asymmetrical Dimethylated Arginine

ADP – Adenosine Diphosphate

AKI – Acute Kidney Injury

AKIN – Acute Kidney Injury Network

AMP – Adenosine Monophosphate

APPLE – Acute Patient Physiologic and Laboratory Evaluation

ARF – Acute Renal Failure

ATP – Adenosine Triphosphate

BP – Blood Pressure

BPM – Breath per minute

BUN – Blood Urea Nitrogen

Cfu – Colony forming units

CKD – Chronic Kidney Disease

CRP – C Reactive Protein

CRRT – Continuous Renal Replacement Therapy

CVP – Central Venous Pressure

ERRT – Extracorporeal Renal Replacement Therapy

FENa – Fractional Excretion of Sodium

GFR – Glomerular Filtration Rate

GGT – Gamma-Glutamyl-Transpeptidase

ICU – Intensive Care Unit

IgG – Immunoglobulin G

IHD – Intermittent Haemodialysis

IRIS – International Renal Interest Society

K^+ – Potassium

MAP – Mean Arterial Pressure

MAT – Microglutination Test

Na^+ – Sodium

NAG – N-acetyl-β-D-glucosaminidase

NGAL – Neutrophil gelatinase associated protein

PCV – Packed Cell Volume

PD – Peritoneal Dialysis

RBF – Renal Blood Flow

RBP – Retinol Binding Protein

RIFLE – Risk, Injury, Failure, Loss, End-stage disease

RRT – Renal Replacement Therapy

SCr – Serum Creatinine

SDMA – Symmetric Dimethylated Arginine

SpO_2 – Peripheral capillary Oxygen Saturation

TP – Total Protein

USG – Urine Specific Gravity

WBC – White Blood Cell

µNAG – Urinary N-acetyl-β-D-glucosaminidase

LIST OF SYMBOLS

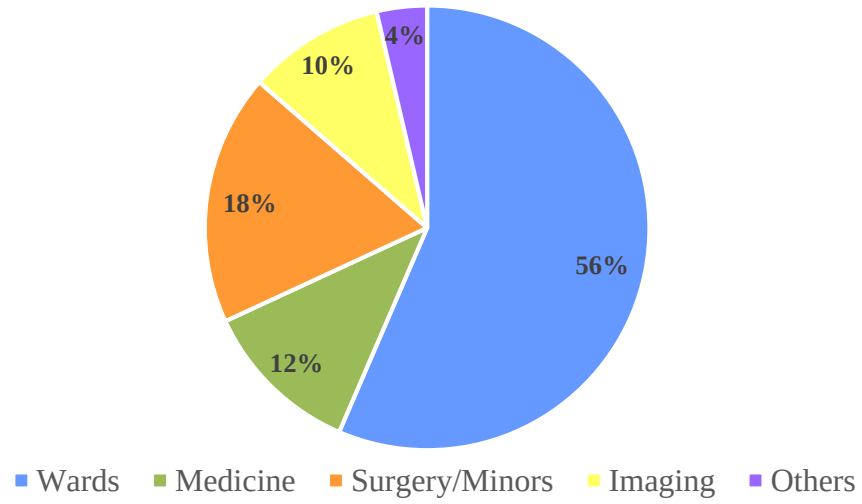
%	percentage
α	alpha
β	beta
g	gram
mg	milligram
l	litre
dl	decilitre
ml	millilitre
mEq	milliequivalent
mmol	millimole
µmol	micromole
ºC	Celsius degrees
mmHg	millimetre of mercury
>	more than
<	less than
≥	more or equal than
≤	less or equal than
=	equal to

PART I – EXTERNSHIP REPORT

The Integrated Masters in Veterinary Medicine requires a training period during the 6th year, which I performed for a 4 month period, starting on September 19th until January 20th, at London Vet Specialist / Village Vet Hampstead /Vet 24, London, under the supervision of Dr Adam Mugford (BVetMed MVetMed DACVECC MRCVS, RCVS Recognised Specialist in Emergency and Critical Care and Honorary Lecturer University of Liverpool School of Veterinary Science) and co-supervised by Professora Doutora Maria Manuela Rodeia Espada Niza (FMV-ULisboa).

During this four months period, I performed approximately 885 hours of training. The hours of training were divided into the different clinical areas (Chart 1) and all the activities I enrolled were realised under the supervision of the veterinarians or veterinary nurses.

Chart 1 – Distribution of time in different clinical areas



Most of my training hours were spent in ward area and intensive care unit; here we would start with morning rounds, where the clinical information about the hospitalized patients was transmitted from the night veterinarian to the veterinarians in charge of the cases during the day. After discussing the cases, I would assist the veterinary nurses with their tasks, such as physical examinations, intravenous (IV) catheter checks, IV catheter placement and removal, bandaging the IV, monitoring critical patients, taking blood samples, setting fluid therapy systems and bags, preparation and administration of IV and oral drugs, urine output monitoring, pain scoring, patient restraining, feeding patients, sometimes via naso-oesophageal or oesophageal feeding tubes, changing beds and cleaning kennels. During my

time in wards I also assisted with the management, examination and monitoring of Specialist Critical Care Patients.

In Surgery, I helped with patient's admission, pre-medication preparation and administration, IV placements, ECG monitoring and set up, monitoring of non-invasive blood pressure, endotracheal intubation, clipping and cleaning the surgical site, monitoring the anaesthesia and vital signs, and post-surgery I would help with patient's anaesthetic recovery. In some surgeries I assisted the surgeon to observe the procedures and help with what was necessary. I performed routine surgeries, as feline and canine castration. I also assisted an American and European and RCVS Board-Certified Surgical Specialist with complex surgery, with anaesthesia being performed by a residency trained (board eligible) anaesthetist. I was also able to assist with dentistry and dental radiography, which were performed by a member of the Australian college of Dentistry and Oral Surgery and RCVS advanced practitioner.

In Medicine, I assisted consultations, were I improved my communication skills and my knowledge on how to conduct a conversation with clients to gain a thorough clinical history. Discussion of the cases, such as symptoms, differential diagnosis, prioritised problem list, imaging findings and therapies, were done during morning and afternoon rounds, if the patient stayed hospitalized or after the consult.

In Imaging, I helped veterinarians and veterinary nurses positioning the patients for radiographs and ultrasounds and could discuss the imaging findings with the clinicians. I was also able to assist to ultrasounds performed by a Board Certified Diagnostic Imaging Specialist.

Other procedures that I assisted were endoscopies, central venous catheterization and urinary catheter placements and naso- and oesophageal feeding tubes placement. Under the supervision of my mentor (a Board-Certified Emergency and Critical Care Specialist) I placed an indwelling thoracic drain via modified (wire guided) seldinger technique under sedation, for drainage of a neoplastic pleural effusion prior to CT imaging.

During my training period, I designed and conducted my prospective study project for my master's degree dissertation, regarding the detection of AKI using IRIS AKI criteria comparing to the creatinine reference range in small animals with a probable renal toxin exposure. My interest on this topic was due the number of cats and dogs hospitalized with possible development of AKI, and that some clinics still use the creatinine reference range to detect AKI instead of the new criteria, the IRIS AKI criteria.

PART II – ACUTE KIDNEY INJURY A LITERATURE REVIEW

2.1 INTRODUCTION

The kidneys have many functions, which are important to maintain homeostasis, including electrolyte, acid-base and water balance regulation, arterial blood pressure regulation, excretion of metabolic wastes, excretion exogenous compounds, as drugs, production of erythropoietin, synthesis of active vitamin D, and gluconeogenesis (Lunn, 2011).

The functional unit of the kidneys is the nephron, which includes the glomerulus, which is interposed between an afferent and efferent arteriole within the renal cortex; the Bowman's capsule and the renal tubule. The different functions of the segments of the renal tube are due to the functional and structural specializations of the epithelial cells of the tube (Lunn, 2011).

The kidneys receive approximately 20% of the cardiac output. The blood enters the glomeruli through the afferent arterioles, 20% of the plasma goes into Bowman's capsule and 80% leaves through the efferent arterioles. Of the blood leaving the glomerulus, 90% or more goes through the peritubular capillaries into the renal cortex, and the remaining 5% to 10% goes into the medulla through the vasa recta. This means that the cortex is especially susceptible to blood toxins and the medulla is more susceptible to ischemia (Lunn, 2011).

The glomerular filtration rate (GFR) is defined as the volume of fluid filtered from the afferent arterioles into the Bowman's capsule per unit of time (Smarick & Hallowell, 2015), and it reflects renal function. GFR is affected by the permeability and hydraulic pressure in the glomerular capillaries, the hydrostatic pressure in Bowman's capsule, and the oncotic pressure of the blood. The kidneys can regulate the GFR apart of the renal blood flow (RBF) thanks to changes in pressure of the arterioles. Changes in RBF affect the metabolic functions and the integrity of the tubules while changes in GFR affect excretion of water and solutes (Lunn, 2011).

In human medicine as well as in veterinary medicine, the term acute renal failure (ARF) was used in patients with an acute decrease in renal function, resulting in the excretion of urea and creatinine, and therefore an increase in serum creatinine above the reference range. However, this term was insensitive to detect patients with mild signs of renal dysfunction, since injury can be difficult to define as 'failure' and therefore this term has been replaced by acute kidney injury (AKI), so the disease can be recognised earlier, since it has been shown that early detection is beneficial to outcome in human medicine (Mugford, Li, & Humm, 2013).

2.2 ACUTE KIDNEY INJURY (AKI)

2.2.1 Definition

AKI is the term used when a rapid loss in renal function occurs (Thoen & Kerl, 2011; Mugford et al, 2013), associated with a sudden decrease in the GFR and changes in urine volume and renal solute excretion (Brown, Segev, Francey, Kass, & Cowgill, 2015; Brown, 2016).

2.2.2 AKI Staging System

In 2004, the Acute Dialysis Quality Initiative Group (ADQI group) developed the first consensus definition for AKI, in human medicine, known as the RIFLE criteria. The RIFLE criteria (Figure 1) divides the patients into risk (R), injury (I), failure (F), loss (L) and end-stage disease (E). These categories are based on increased serum creatinine concentrations, decreased GFR or decreased urine output. In 2007, the Acute Kidney Injury Network (AKIN) published the AKIN criteria (Figure 1), which represents an even broader spectrum of renal dysfunction. In this consensus, the stages are also based on increased serum creatinine or decreased urine output, but do not include the GFR (Thoen & Kerl, 2011).

In 2012, the Kidney Disease: Improving Global Outcomes (KDIGO) developed a new staging system for AKI (Figure 1), in which AKI is defined as an increase in SCr by $\geq$ 0.3 mg/dl ($\geq$ 26.5 µmol/l) within 48 hours; or an increase in SCr to $\geq$ 1.5 times baseline, which is known or presumed to have occurred within the prior 7 days; or urine volume < 0.5 ml/kg/h for 6 hours ("KDIGO Clinical Practice Guideline for Acute Kidney Injury", 2012).

Koeze et al, (2017) analysed differences in AKI incidences based on the definitions of RIFLE, AKIN and KDIGO, each with and without urine output. The results showed that urine output criteria increased the incidence of AKI by adding the less sick patients and enabled a sooner recognition of AKI by 11 hours, especially in patients with mild to moderate AKI. RIFLE creatinine definition requires a higher creatinine rise than the criterion on AKIN and KDIGO definitions, and therefore patients will meet more easily the AKIN and KDIGO criteria, enabling AKIN and KDIGO criteria to detect patients with AKI with greater sensitivity compared to RIFLE criteria (Koeze et al, 2017).

Figure 1 - Comparison between AKI grading systems (Adapted from Elberry, 2015)

RIFLE criteria (2004)	AKIN criteria (2007)	KDIGO criteria (2012)
Definition of AKI		
Abrupt (1-7 days) ⬇ in renal function from baseline	Abrupt (within 48h) ⬇ in renal function from baseline	Abrupt (hours – days) ⬇ in renal function from baseline
Definition of decrease in renal function		
- ⬆ in SCr of 0.5 mg/dl or more or - ⬆ in SCr **1.5** – fold from baseline or - ⬇ UO < 0.5 ml/kg/h for > 6h or - ⬇GFR > 25%	- ⬆in SCr of 0.3 mg/dl or more or - ⬆ in SCr **1.5**-fold from baseline or - ⬇ UO < 0.5 ml/kg/h for > 6h	- ⬆in SCr of 0.3 mg/dl or more within 48h or - ⬆ in SCr **1.5**-fold from baseline within the last 7 days or - ⬇ UO < 0.5 ml/kg/h for > 6h

In veterinary medicine, the International Renal Interest Society (IRIS) developed a consensus for staging Chronic Kidney Disease (CKD), to promote a more uniform characterization and recognition of the disease. But there was no definition or staging scheme adopted for AKI and therefore, in 2013 IRIS produced a similar staging system to the CKD staging system to classify and grade the severity of AKI in dogs and cats, based upon the work of others (Lee et al, 2011; Thoen & Kerl, 2011). This grading system allows an earlier recognition and outcomes assessment of AKI and is divided into five grades, which are based on blood creatinine and urine output (Figure 2) (Brown, 2016) . Although this grading system has not yet been validated for use in general practice.

Figure 2 – IRIS AKI grading criteria (Adapted from Brown, 2016)

AKI Grade	Blood Creatinine	Clinical Description
Grade I	<1.6 mg/dl (<140 µmol/l)	Nonazotemic AKI: a. Documented AKI: (historical, clinical, laboratory, or imaging evidence of AKI, clinical oliguria/anuria, volume responsiveness‡) and/or b. Progressive nonazotemic increase in blood creatinine: ≥ 0.3 mg/dl (≥ 26.4 µmol/l) within 48 h c. Measured oliguria (<1 ml/kg/h)# or anuria over 6 h
Grade II	1.7 – 2.5 mg/dl (141 – 220 µmol/l)	Mild AKI: a. Documented AKI and static or progressive azotemia b. Progressive azotemic: increase in blood creatinine; ≥ 0.3 mg/dl ≥ 26.4 µmol/l) within 48 h),or volume responsiveness‡ c. Measured oliguria (<1 ml/kg/h)# or anuria over 6 h
Grade III	2.6 – 5.0 mg/dl (221 – 439µmol/l)	Moderate to Severe AKI: a. Documented AKI and increasing severities of azotemia and functional renal failure
Grade IV	5.1 – 10.0 mg/dl (440 – 880 µmol/l)	
Grade V	>10.0 mg/dl (>880 µmol/l)	

(‡Volume responsive is an increase in urine production to >1 ml/kg/h over 6 h; and/or decrease in serum creatinine to baseline over 48 h)

All grading categories are then sub graded based on urine production as oligoanuric (O; oliguria, < 1 ml/kg/h, or anuria, no urine produced, over 6 hours) or nonoliguric (NO; > 1 ml/kg/h) and on the requirement for renal replacement therapy (RRT) (Figure 3) (Brown, 2016)

Figure 3 – IRIS AKI sub grading criteria (Adapted from Brown, 2016)

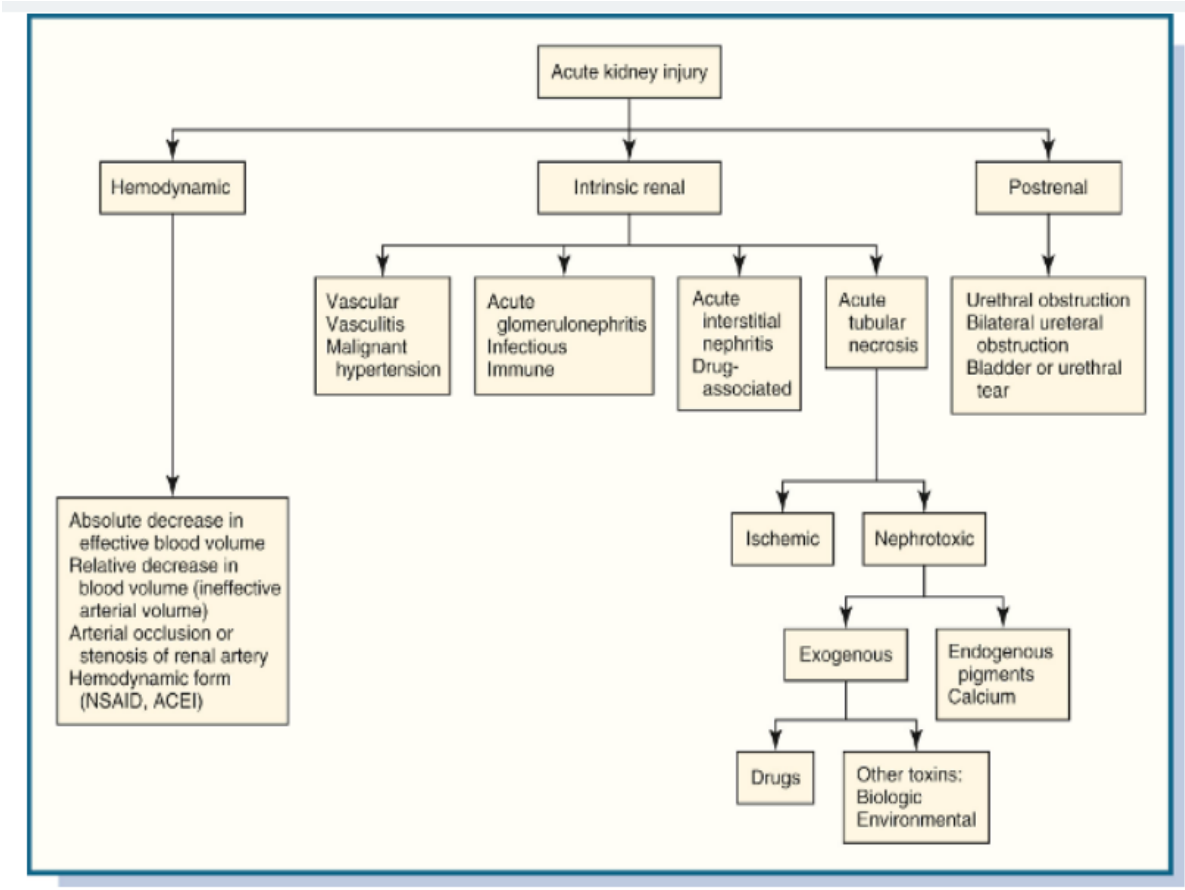
AKI Grade	Blood Creatinine	Subgrade
Grade I	<1.6 mg/dl (<140 µmol/l)	Each grade of AKI is further subgraded as: 1. Non oliguric (NO) or oligo-anuric (O) 2. Requiring renal replacement therapy (RRT)
Grade II	1.7 – 2.5 mg/dl (141 – 220 µmol/l)	
Grade III	2.6 – 5.0 mg/dl (221 – 439µmol/l)	
Grade IV	5.1 – 10.0 mg/dl (440 – 880 µmol/l)	
Grade V	>10.0 mg/dl (>880 µmol/l)	

2.2.3 Aetiology of AKI

There are several causes of AKI, which are classified into hemodynamic or pre-renal, intrinsic, and post-renal (Figure 4) (Langston, 2017). Azotaemia is defined as biochemical change induced by all categories of causes for AKI, and corresponds to an increase in serum creatinine and/or blood urea nitrogen (BUN) concentrations (Lunn, 2011; Langston, 2017). Uraemia is defined as the presence of urine constituents in the blood, such as urea and other nitrogenous waste compounds, however it is usually used to refer the clinical signs that develop as azotaemia worsens (Lunn, 2011).

In human patients in ICU, the most frequent causes for AKI are sepsis, surgery, low cardiac output, hypovolemia and medications, and therefore it is reasonable to assume that these are also important causes for AKI in veterinary patients (Lunn, 2011). In dogs, the major causes for AKI are infectious diseases, toxic insults, hemodynamic instability, immune-mediated diseases and miscellaneous and idiopathic aetiologies. In many diseases states the presence of AKI increases the patient risk of mortality (Brown et al, 2015).

Figure 4 – Categories of AKI (Adapted from Langston, 2017)



2.2.3.1 Pre-renal AKI

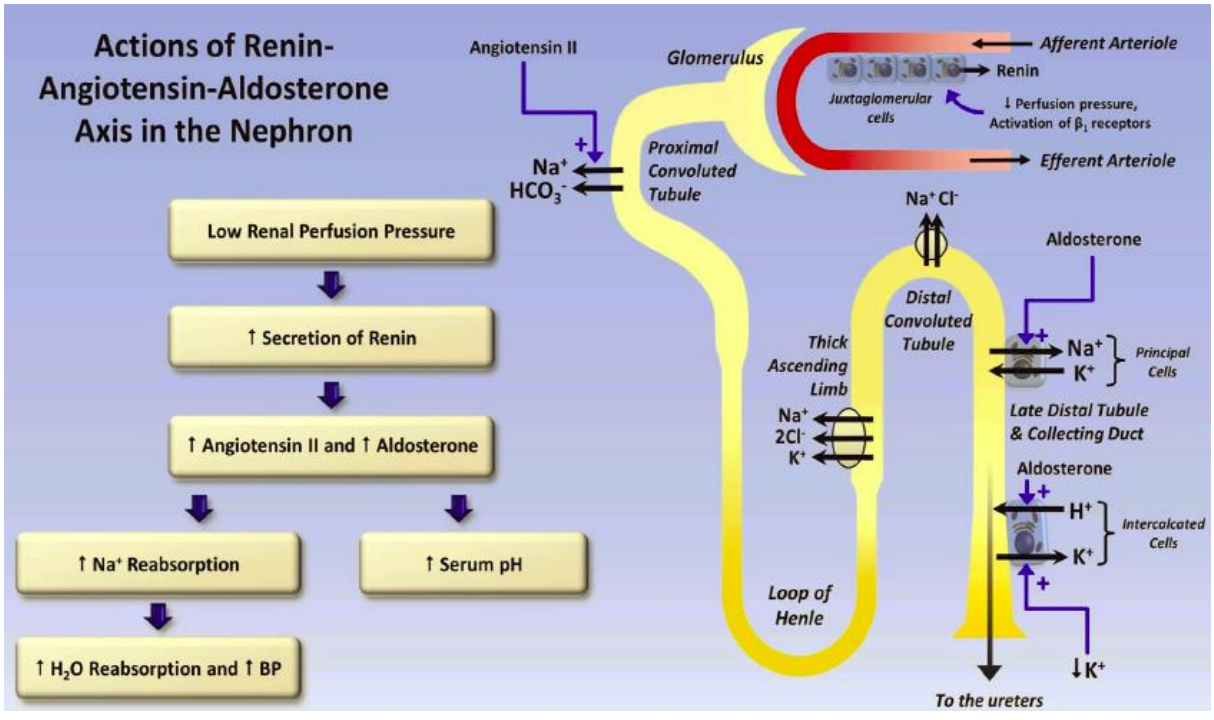
The term pre-renal implies normal renal morphology with a decrease in GFR due to a decrease in RBF. Renin is released in response to renal hypoperfusion with consequent increase in angiotensin II, which causes arterial constriction, and aldosterone, responsible for the absorption of Na^+ and H_2O in the distal nephron (Figure 5). A decrease in GFR may occur due to decreased cardiac output, hypovolemia, hypotension, dehydration, anaesthesia, hypoalbuminemia (decreased plasma oncotic pressure) increased blood viscosity, occlusion or constriction of the renal artery, trauma, surgery or shock (hypovolemic, haemorrhagic, hypotensive, septic), or hypoadrenocorticism (Lunn, 2011; Harison, Langston, Palma & Lamb, 2012; Langston, 2017). This term also includes an increase in BUN and creatinine concentrations and a concentrated urine specific gravity (USG) (Lunn, 2011; Langston, 2017). A pre-renal azotaemia should be considered if the patient has a history of excessive fluid loss or decreased fluid intake. Serum creatinine levels are dependent on GFR, since creatinine is filtered at the glomerulus, and it is not significantly secreted or reabsorbed, while urea is secreted and reabsorbed, as well as freely filtered. Therefore, when GFR decreases due to pre-renal factors, both creatinine and BUN increase (Lunn, 2011).

USG greater than 1.030 in a dog or 1.035 in a cat indicates a pre-renal cause (Syme & Jepson, 2017), and therefore patients should be assessed for signs of dehydration or decreased perfusion, and these parameters should be corrected. The fractional excretion of sodium (FE_{Na}), corresponds to the percentage of sodium filtered by the kidney and excreted in the urine, and it can be used to help differentiate pre-renal azotaemia from intrinsic renal azotaemia. FE_{Na} can be calculated with the equation $\frac{(Urine\ Na\ x\ Plasma\ creatinine)}{(Plasma\ Na\ x\ Urine\ creatinine)}$ x 100. In pre-renal azotaemia with volume depletion, the kidneys conserve sodium and the fractional excretion is < 1%. In intrinsic renal azotaemia the FE_{Na} is higher (Langston, 2017).

In concurrent disease processes, as hypoadrenocorticism and hypercalcaemia, USG may not allow the identification of pre-renal azotaemia, due to an inappropriate urine concentration despite the kidneys functioning adequately (Mugford et al, 2013).

In most cases the choice of therapy is the restoration of perfusion with intravenous fluids. However, patients with primary cardiac disease with reduced contractility or sepsis do not respond to IV fluids (Langston, 2017).

Figure 5 – Actions of Renin-Angiotensin-Aldosterone in the nephron (Adapted from Strong, 2013)



2.2.3.2 Intrinsic AKI

Intrinsic AKI implies that the kidney is impaired and unable to function properly (Lunn, 2011). There are several causes of intrinsic AKI (Table 1), such as ischemic events, primary renal diseases, secondary disease with renal manifestation and nephrotoxins (Table 2).

Urine specific gravity (USG) can be used to help determine whether the patient has intrinsic renal azotaemia, usually when the value is between 1.008 and 1.015.

Table 1 – Causes of intrinsic AKI (Adapted from Langston, 2017)

ISCHEMIC EVENTS	PRIMARY RENAL DISEASES	SECONDARY DISEASES WITH RENAL MANIFESTATION	NEPHROTOXICANTS
- Shock (hypovolemic, hemorrhagic, hypotensive, septic) - Decreased cardiac output (congestive heart failure, arrhythmias, cardiac arrest, cardiac tamponade) - Deep anaesthesia/ extensive surgery - Trauma - Hyperthermia/ hypothermia - Extensive cutaneous burns - Transfusion reaction - Renal vessel thrombosis/ DIC - Hyperviscosity/ polycythemia - NSAIDs - Hypercalcaemia	- Infectious (pyelonephritis, leptospirosis, borreliosis) - Immune-mediated (acute glomerulonephritis, SLE, renal transplant rejection, vasculitis) - Neoplasia (lymphoma)	- Infectious (feline infectious peritonitis, babesiosis, leishmaniasis, bacterial endocarditis) - Systemic inflammatory response syndrome, sepsis, multiple organ failure, disseminated intravascular coagulopathy - Pancreatitis - Hepatorenal syndrome - Malignant	- Exogenous toxins - Drugs - Endogenous toxins

Table 2 – Nephrotoxicants (Adapted from Mugford et al, 2013)

Antimicrobials	**Antiprotozoals**	Superphosphate fertiliser
Aminoglycosides	TMPS	
Cephalosporins	Thiacetarsamide	**Miscellaneous toxins**
Penicillins	Pentamidine	**Lilies (all parts of the plant, although Arum/Calla lilies are non-toxic)**
Sulfonamides	Dapsone	
Quinolones		Grapes, raisins and sultanas
Tetracyclines	**Antifungal drugs**	Vitamin D intoxication (psoriasis cream or rodenticide)
Vancomycin	Amphotericin B	
Carbapenems		Vitamin D3 analogue
Polymyxin B	**Antiviral drugs**	Cortinarius mushrooms
Rifampin	Aciclovir	Snake envemonation
TMPS	Foscarnet	Bee sting
NSAIDs	**Radiocontrast agents**	**Endogenous toxins**
All	Ionic contrast medium	Haemoglobin
	High-osmolality contrast media	Myoglobin
ACE inhibitors		
All	**Calcium antagonists**	**Heavy metals**
	Bisphosphonates	Mercury
Diuretics	Galium nitrate	Lead
All		Bismuth salts
	Organic compounds	Copper
Chemotherapeutic agents	**Ethylene glycol**	Nickel
Cisplatin	Chloroform	Silver
Carboplatin	Pesticides	Gold
Doxorubicin	Herbicides	Chromium
Methotrexate	Solvents	Arsenic
	Carbon tetrachloride and other chlorinated hydrocarbons	
Immunosuppressive drugs		
Cyclosporine		
Azathioprine		

ACE Angiotensin-converting enzyme, NSAID Non-steroidal anti-inflammatory drug, TMPS Trimethoprim-sulfamethoxazole
Bold, blue type indicates most common

2.2.3.3 Post renal AKI

Post renal azotaemia is associated with an increase in BUN and creatinine concentrations, because urine does not exit through the normal route, which can occur due to obstruction in the urinary tract, or rupture of the urinary tract with leakage of urine (Lunn, 2011). The most common causes of ureteral obstruction is lithiasis, trigonal neoplasia, ureteral strictures, blood clots and other neoplastic causes (Langston, 2017).

Post renal azotaemia, as urethral obstruction or uroabdomen, can be identified by palpating the bladder and diagnostic imaging to check if there is abdominal free fluid. In case of uroabdomen the free fluid has high concentrations of potassium, urea and creatinine compared with serum concentrations (Mugford et al, 2013).

2.2.3.4 Hospital-acquired AKI

There are conditions that may predispose the patient to develop AKI along with poor decisions made by the clinician that can enhance the side effects of certain drugs. For instance, patients presented in shock have a decreased renal perfusion and decreased renal blood flow due to the vasoconstriction caused by angiotensin II and noradrenaline. Although, intrinsic prostaglandins prevent excessive reduction in renal blood flow, drugs such as NSAIDs or other nephrotoxic medications, should not be used (Mugford et al, 2013).

Cardiac disease can lead to poor renal perfusion, which can be worsened by used medications as angiotensin-converting enzyme (ACE) inhibitors, loop diuretics and carbonic anhydrase inhibitors. Therefore, animals with cardiac disease or with pre-existing chronic kidney disease (CKD) and patients with low urine output are more likely to develop hospital-acquired AKI (Mugford et al, 2013).

AKI may occur in animals with pre-existing CKD, due to a rapid reduction of renal function, being this known as acute on chronic kidney disease (AOCKD). This usually occurs due to complications of CKD, or underlying causes such as pyelonephritis, urethral obstruction, chronic interstitial nephritis with acute tubular necrosis, within others (Mugford et al, 2013).

2.2.4 Phases of AKI

AKI is frequently described in four stages. The initiation phase, correspond to the period of exposure, in which the insult to the kidneys occur. During this phase, the renal blood flow decreases and the obstruction of the renal tubules caused by casts lead to a decrease in the GFR. The following stage is the extension phase, that can last for 48-72 hours, in which occur ischemia, inflammation and cellular injury, leading to cellular apoptosis, necrosis, or both (Lunn, 2011; Ross, 2011; Mugford et al, 2013). During extension phase the accumulation of renal analytes begins rapidly. The third stage or maintenance phase, can last for 7 to 21 days and is when usually AKI is detected due to an increase in creatinine and urea (Ross, 2011; Mugford et al, 2013) . In this phase, the increase loss of sodium causes a greater amount of chloride to reach the macula densa, resulting in a worst ischaemia, due to the constriction of the afferent arterioles. It is usually in this phase that anuria develops. The last stage is recovery, in which azotaemia improves and renal tubules repair (Lunn, 2011; Ross, 2011; Mugford et al, 2013). Renal function may return to normal or it can occur scar formation leading to long term kidney dysfunction (Ross, 2011; Mugford et al, 2013;).

2.2.5 Mechanisms of pathophysiology

The rapid loss in renal function that occurs with AKI leads to a decrease in RBF and cellular damage (Lunn, 2011; Ross, 2011). Ischemia is responsible for the degradation of intracellular adenosine triphosphate (ATP) to adenosine diphosphate (ADP) and adenosine monophosphate (AMP), leading to an energy deficit. The decrease in ATP causes an increase in intracellular calcium, and a decrease activity of Na^+K^+-ATPase pump, which will alter the concentration gradient of Na^+ and K^+. This results in movement of water into the cell causing cell swelling which may cause tubular obstruction (Ross, 2011; Langston, 2017). This loss in polarity alters the movement of the solutes and the redistribution of the Na^+K^+-ATPase pump, from the basolateral plasma membrane to the apical cell membrane, altering the proximal tubular sodium management, which results in an increased fraction of filtered sodium to the macula densa (Lunn, 2011; Ross, 2011; Langston, 2017). As result the feedback leads to afferent arteriolar constriction resulting in a decreased GFR (Ross, 2011).

The production of peroxynitrite due to ischemia can inhibit renal tubular cell matrix attachment which will delay the tubular epithelial regeneration (Ross, 2011). Ischemia also causes integrins to redistribute from the basal to the apical tubular cell membrane, resulting in a loss of support of tubular cells to the membrane and cell desquamation. Desquamated cells may adhere to the apical cell membrane of intact tubular cells, due to the integrin receptors, leading to tubular obstruction (Ross, 2011; Langston, 2017).

Neutrophil activation occurs as a response of inflammation. Neutrophils migrate into the cells interstitium, altering the renal tubular cell integrity. Therefore, neutrophil accumulation as well as platelets and red blood cells may cause capillary pugging (Ross, 2011).

In AKI, necrosis occurs when there is ischemia or toxic injury. Although, apoptosis of renal tubular cells may also occur it appears that this happens when the insults are less severe, whereas those that are more severe lead to necrosis (Ross, 2011). The recovery depends on regeneration of the injured cells and normalizing its polarity, migration of viable cells, and removal of necrotic cells and plugs (Langston, 2017).

2.3 DIAGNOSIS OF AKI

2.3.1 History and Physical examination

Patients with AKI often have non-specific symptoms as anorexia, vomiting, lethargy and diarrhoea. Signs such as polydipsia, polyuria, anuria, ataxia, dyspnoea, syncope and seizures have also been reported (Mugford et al, 2013).

Animals with exposure to nephrotoxins may have significant historical findings, such as vomiting of plant parts after lillie ingestion in cats, or acute central nervous system signs in ethylene glycol toxicosis in dogs and cats (Langston, 2017).

According to Mugford et al (2013), in veterinary studies of patients presenting AKI, the most common findings are mild to severe dehydration, tachycardia (caused by pain or hypovolemia), bradycardia (due to hyperkalaemia), enlarged painful kidneys, pyrexia (caused by pyelonephritis) and halitosis. However, some patients may have no signs at all (Figure 6).

Figure 6 – Differences between patients with AKI and CKD (Adapted from Mugford et al, 2013)

Common findings on initial presentation	Acute kidney injury	Chronic kidney disease
Body condition score	Normal	Decreased
Packed cell volume	Normal	Reduced (non-regenerative anaemia)
Renal size	Normal/increased	Normal/reduced
Thirst	Decreased/normal	Increased
Urine output	Decreased/increased	Increased
Abdominal pain	Present	Absent
Uraemic ulceration	Initially absent	Present
Soft tissue mineralisation	Absent	Present (end stage)

2.3.2 Laboratory evaluation

Critical patients need to be monitored closely and therefore a packed cell volume (PCV), a total protein (TP), a venous blood gas analysis and electrolytes is recommended. Although serum creatinine concentration is not a sensitive marker of kidney function, in patients with AKI, creatinine should be included, to apply the IRIS AKI criteria (Ross, 2011; Harison et al, 2012; Mugford et al, 2013).

Patients with AKI are likely to have electrolytes disturbances. While anuric and oliguric patients are often presented with hyperkalaemia, polyuric patients can have hypokalaemia (Ross, 2011; Mugford et al, 2013;). Potassium concentrations greater than 6mmol/l may cause cardiac dysfunction and concentrations below 2.5 mmol/l may cause muscle weakness including respiratory muscle depression (Mugford et al, 2013). Both hyper and hyponatremia can occur. In ethylene glycol toxicity, acute signs such as nausea, vomiting and ataxia due to plasma hyperosmolality, appear in the first 30 minutes to 12 hours. After this time, the patient seems to recover only till the clinical signs corresponding to the next phase appear (Hovda, Brutlag, Poppenga & Peterson 2016). The measurement of the ionised or total calcium may help with the diagnosis, since it chelate to form calcium oxalate when in contact with the toxicant, which causes hypocalcaemia, which can be severe enough to cause cardiovascular

depression (Ross, 2011; Mugford et al, 2013;). Alterations in ECG, occur after 12-24 hours in cats and 36-72 hours in dogs. Usually anuric renal failure develops 72-96 hours post ingestion (Hovda et al, 2016).

Blood gas analysis can be very informative, since these patients often have severe metabolic acidosis due to the decreased renal excretion of acid. A pH less than 7.1 will cause cardiovascular depression and damage the enzyme pathways (Mugford et al, 2013).

A blood smear can be done in order to help support certain diagnosis such as haemoglobinaemia as the possible cause for the AKI (Mugford et al, 2013).

A serum biochemical profile may indicate an aetiology for AKI such as feline infectious peritonitis or multiple myeloma, if hyperglobulinaemia is seen (Mugford et al, 2013). Diseases as leptospirosis may be suspected if alterations in alanine aminotransferase, alkaline phosphatase, α-glutamyl transferase and total bilirubin occur (Mugford et al, 2013).

2.3.2.1 Urinalysis

Urinalysis and urine sediment examination can provide evidence of renal disease before changes in serum creatinine or urine output occur, such as pyuria, bacteriuria and the presence of glycosuria in the absence of hyperglycaemia (Lunn, 2011).

Cystocentesis should be performed before fluid therapy, since urinalysis gives important information in all cases of suspected AKI. Although, fluid therapy should not be delayed in unstable patients. Usually these patients have isosthenuric urine with a USG between 1.007 and 1.015. Urinary tract infections are likely when in a catheterised sample there are more than 10^3 colony-forming-units (cfu)/ml in cats and male dogs and more than 10^5 cfu/ml in female dogs with a urinary pathogen (Mugford et al, 2013). The RapiBac™ Vet is a lateral flow immunoassay for the detection of Gram-positive and Gram-negative bacteria in urine, which uses monoclonal antibodies to directly detect bacteria in urine at concentrations as low as 1000 cfu/ml. The advantages of this test are results in less than twenty minutes, costs less than urine cultures and does not require instrumentation(http://www.rapidbacvet.com/, 2016).

Sediment analysis could show cristalluria, such as monohydrate calcium oxalate crystals which is compatible with ethylene glycol toxicity (Mugford et al, 2013).

2.3.3 Tests for specific diseases

Leptospirosis is usually diagnosed by detecting antibodies using a microagglutination testing (MAT). Antibodies can be formed after a subclinical infections and vaccination leading to false positives, therefore only values greater than 1:800 are strongly suggestive of infection. If

suspicious is strong, despite of lower titres present, a second MAT should be performed within 2 to 4 weeks (Sykes et al, 2011; Mugford et al, 2013; Schuller et al, 2015).

In ethylene glycol toxicity, urine tests or serum ethylene glycol measurements are available, but if drugs with propylene glycol such as activated charcoal products or injectable diazepam has been given to the patient, false positives may occur (Ross, 2011; Mugford et al, 2013).

2.3.4 Imaging

Renal size can be assessed via radiography. The normal kidney in cats has a length of 2.4 to 3.0 times the height of the second lumbar vertebrae, and in dogs has 2.5 to 3.5 times the height (Mugford et al, 2013). Post renal azotaemia can be diagnosed if radio-opaque nephroliths, ureteroliths or urethroliths are detected (Ross, 2011; Mugford et al, 2013).

Ultrasonography can be used to assess the renal pelvis and the renal parenchyma. Dilated renal pelvis can be seen in patients with pyelonephritis, volume overload, those treated with higher-rate fluid therapy or diuretics, or those with ureteric obstruction. Shrunken kidneys with hydronephrosis can indicate chronic or end stage disease. Ethylene glycol exposure can be suspected if the renal echogenicity is increased related to the liver and there is a presence of the 'halo' sign, which corresponds to an increase in cortical and medullary echogenicity (Mugford et al, 2013). Abnormal subcapsular fluid accumulation can occur in inflammation, infection, toxicity or neoplasia (Ross, 2011). Post renal azotaemia due to urinary tract rupture can be diagnosed when the retroperitoneal and peritoneal spaces are assessed for free fluid. Free fluid can also appear in patients with fluid overload. Lymphoma can be diagnosed with a fine-needle aspiration of the kidneys in the presence of renomegaly and compatible ultrasonography signs (Mugford et al, 2013).

2.4 TREATMENT OF AKI

2.4.1 Specific therapies

An early intervention may prevent the occurrence or the progression of AKI. Therefore, in animals with a recent history of toxicants ingestion, such as ethylene glycol, or of toxins ingestion, such as grapes or raisins (dogs) or lilies (cats), emesis should be induced as long as the patient is awake enough to protect its airways, otherwise aspiration pneumonia may occur (Ross, 2011; Li, Mugford, & Humm, 2013).

In patients presenting a history of ethylene glycol toxicity, ethanol or 4-methylprazole (4-MP) should be used, since the alcohol dehydrogenase has a preference for those substances instead and therefore the ethylene glycol is not metabolized and toxication does not occur. Although it is most effective within six to eight hours of ingestion (Li et al, 2013). Li et al (2013) state

that a recent study showed that it's safe and effective to give high doses of 4-MP in cats within three hours of ingestion to prevent fatal renal failure. Intravenous fluid diuresis can improve the renal excretion of intact ethylene glycol and haemodialysis can remove not only the intact ethylene glycol but also its metabolite glycolic acid (Ross, 2011), but once azotaemia develops it is fatal.

Nephrotoxic medications should be discontinued immediately, if the diagnosis of AKI is established (Li et al, 2013). Empiric therapy with broad-spectrum antibiotics that concentrate in urine, such as amoxicillin-clavulanate or ampicillin, should be administered intravenously until pyelonephritis is ruled out via negative urine culture. In geographic areas where leptospirosis occurs, dogs that do not have any other evidence of another AKI cause should receive antibiotics that target *Leptospira* species while results are pending (Ross, 2011; Li et al, 2013). Intravenous antibiotics, as ampicillin or amoxicillin are usually used to reduce multiplication, shedding and transmission of the leptospiraemia. Oral tetracyclines, as doxycycline, or fluoroquinolones are used for elimination of the carrier state (Li et al, 2013).

2.4.2 Supportive therapies

Fluid therapy should be used to correct fluid deficits, electrolyte imbalances and acid-base disorders, since these are the mainstays for treating AKI. Although fluid therapy has been used in high rates to increase the RBF and the GFR, this does not always result in increased urine production and can cause fluid overload, especially in patients with oliguria or anuria (Ross, 2011; Li et al, 2013). According to Li et al (2013), fluid overload has been associated with increased mortality in humans with AKI. However, in patients presenting hypovolemia, which can be due to hypodipsia, vomiting, diarrhoea or third-space losses secondary to hypoproteinaemia or vasculitis due to sepsis, liver failure, protein-losing nephropathy or enteropathy, a high-rate fluid therapy is appropriate (Li et al, 2013).

Inadequate RBF and GFR due to hypovolemia can lead to further renal injury and therefore intravascular volume deficits need to be replaced within 4 to 6 hours with isotonic crystalloids and/or synthetic colloids. In patients with murmurs or diagnosed cardiac diseases replacement should be done over a longer period. Frequent monitoring in all patients is essential to assess response to volume resuscitation and for making appropriate adjustments in therapy, including clinical assessment to patient's mentation, capillary refill time, heart and respiratory rate, pulse quality, arterial blood pressure, packed cell volume and plasma total solids, and serum chemistry parameters, such as BUN, creatinine, sodium, potassium, chloride and phosphorus (Ross, 2011; Li et al, 2013).

Once hypovolemia is corrected, fluid therapy should aim to provide the patient's daily maintenance, and any losses that may occur, such as vomiting or diarrhoea (Ross, 2011; Li et al, 2013).

Fluid balance can be assessed more precisely if urine output is measured, which is best done with an indwelling urinary catheter (Ross, 2011; Li et al, 2013). Li et al (2013) say that his also allows early detection of anuria, oliguria and polyuria.

2.4.3 Management of oliguric or anuric renal failure

Patients with AKI may have a lot of complications. In case of oliguric or anuric patients with AKI, fluid overload is a common complication (Li et al, 2013). Oliguria occurs when the urine output is less than 1 to 2 ml/kg/hour in a well-hydrated patient. When fluid overload occurs the fluid therapy should be stopped until this resolves and the patients should be monitored closely since it has been shown that over hydration worsens the outcome. Li et al (2013) state a valuable tool in guiding fluid therapy in patients with AKI, the central venous pressure (CVP), which measures the hydrostatic pressure within the intrathoracic vena cava and approximately equals right atrial pressure. CVP can be valuable, since normal range is 0 to 5 cm H_2O and values higher than 10 cm H_2O can be suggestive of fluid overload. Unfortunately, CVP correlates poorly with volume status and volume responsiveness (Marick, Baram & Vahid, 2008). Once hydration and volume deficits have been corrected, drug therapy (Figure 7) is the next step to increase urine production, in case oliguria or anuria does not resolve (Li et al, 2013).

Figure 7 – Therapies to increase urine production (Adapted from Li et al, 2013)

Drug	Route	Class	Recommended protocol	Special considerations
Furosemide	IV	Loop diuretic	Loading dose of 0.5 to 1.0 mg/kg IV. Can be increased if no effect seen in 30 to 60 minutes. Can then be followed by 0.5 to 1.0 mg/kg/hour CRI IV or intermittent boluses every eight hours	Significant increase of urine production with no effects on GFR and RBF
Mannitol 20 per cent	IV	Osmotic diuretic	0.5 to 1.0 g/kg over 15 to 20 minutes every four to six hours OR 1 to 2 mg/kg/minute CRI	Infusion should be discontinued if no beneficial effect is seen within one hour Contraindicated in anuric patients or oliguric patients with fluid overload
Dopamine	IV	Vasoactive catecholamines	0.5 to 3 µg/kg/minute CRI	Little to no documented efficacy in dogs and cats with AKI Routine use not recommended

AKI Acute kidney injury, CRI Continuous rate infusion, GFR Glomerular filtration rate, IV Intravenous, RBF Renal blood flow

2.4.3.1 Furosemide

Furosemide is a drug with diuretic effects that inhibits the sodium-potassium-chloride cotransporter located in the apical membrane of the renal tubular cells of the loop of Henle. Diuretic and natriuretic effects of the drug depend on its delivery to the nephrons, which depends on GFR, RBF and active secretion by renal tubular cells on the luminal side (Li et al, 2013).

According to Li et al (2013), the use of furosemide in human patients with AKI is controversial, due to several clinical trials performed that have not shown consistent results in the effectiveness of presenting renal blood flow or improving outcome. Since several veterinary studies found similar results, the benefit of using furosemide in patients with AKI may rely on its diuretic effect, which increases the urine output and therefore allowing the IV fluid therapy to correct acid-base and electrolytes imbalances to continue (Ross, 2011), which may be important in veterinary patients comparing to humans, due to the reduced access to dialysis and costs associated with it.

2.4.3.2 Mannitol

Mannitol is a sugar alcohol that elevates plasma osmolality resulting in expansion of the intravascular volume. It is also an osmotic diuretic as its filtered freely through the glomeruli and it's not absorbed by the renal tubules (Li et al, 2013). Mannitol may also have other beneficial effects in addition to its action as a diuretic, since it has been shown to cause natriuresis by stimulating the release of atrial natriuretic peptide or inhibiting sodium and water reabsorption in the collecting ducts of the nephron, causing more diuresis (Ross, 2011; Li et al, 2013). Other benefits of this drug are renal arteriole dilation, decreased vascular resistance and blood viscosity and acts as a free radical scavenger, reducing oxygen free radicals (Li et al, 2013). It also seems to increase RBF and improve filtration fraction and renal oxygenation in patients with postoperative AKI (Bragadottir, Redfors & Ricksten, 2012).

In oliguric and anuric patients, Mannitol cannot be properly excreted by the kidneys, and therefore this drug is contraindicated in patients with volume overload as it can result in increased serum osmolality, circulating blood volume and blood pressure, leading to pulmonary oedema and congestive heart failure (Ross, 2011; Li et al, 2013). Mannitol is known to be nephrotoxic at higher doses, >2g/kg.

2.4.3.3 Dopamine

Dopamine is a catecholamine neurotransmitter produced from L-tyrosine (Li et al, 2013). Dopamine stimulates two types of dopamine receptors, which are DA-1 and DA-2 and also α and β-adrenergic receptors (Ross, 2011). Activation of DA-1 and DA-2 receptors leads to vasodilation of the interlobular arteries, afferent arterioles and, to a lesser extent, efferent arterioles, resulting in increased GFR and RBF (Li et al, 2013).

Human studies have shown that low-dose dopamine does not have a role in the prevention or treatment of AKI. Although dopamine increases urine output on the first day, it has no effects on renal function (Ross, 2011; Li et al, 2013;). Studies in veterinary patients are limited and there is little documentation on the efficacy of dopamine in dogs and cats with AKI and therefore, Li et al (2013) do not recommend the routine use of dopamine infusion in oliguric or anuric AKI patients.

2.4.3.4 Fenoldopam

Fenoldopam is a selective DA-1 agonist, known to be renoprotective in humans (Gillies, Kakar, Parker, Honoré & Ostermann, 2015). According to Li et al (2013), canine and feline experimental models have been performed and the results show that fenoldopam can induce diuresis. Clinical trials have shown efficacy in normal dogs and delayed but significant efficacy in healthy cats, although no benefits was shown in a clinical study of AKI in dogs and cats (Nielsen, Bracker & Price, 2015).

2.4.3.5 Electrolyte and acid-base abnormalities

Patients with AKI may develop electrolyte and acid-base abnormalities that need to be corrected and monitored otherwise it may become life-threatening. It is common for these patients to develop hyperkalaemia and therefore it is necessary to ensure adequate urine output. If worsening hyperkalaemia is refractory to fluid therapy alone, other therapies can be employed, such as insulin, which shifts potassium into the cells; dextrose, which can be used alone or combined with insulin, since its mechanism is to stimulate endogenous insulin release; β_2-adrenergic agonists, such as terbutaline, also drive potassium into the cell by increasing the activity of the Na^+K^+ ATPase. Sodium bicarbonate also moves serum potassium into the cells, but it is less effective than insulin or β_2-adrenergic agonists (Odunayo, 2014). Other complications that may occur are hypoglycaemia, hypernatremia and respiratory/paradoxical acidosis and volume overload (Li et al, 2013).

In polyuric patients, most likely to occur during recovery phase of AKI, hypokalaemia and hypernatremia can be encountered (Li et al, 2013). Hypokalaemia requires potassium

supplemented fluids (Li et al, 2013). Signs of hypernatremia are nonspecific and usually associated with CNS, including lethargy, weakness, behaviour changes, ataxia, seizures, stupor and coma. Plasma concentrations greater than 170 to 175 mEq/L can cause fluid to shift to extracellular space, causing a decrease in cerebral cellular volume, which can lead to vascular rupture with cerebral bleeding, subarachnoid hemorrhage, permanent neurologic damage, and death. If hypernatremia develops acutely, clinical signs can be present at lower concentrations (Odunayo, 2013). In patients that develop hypernatremia rapidly, sodium concentrations can be corrected rapidly with appropriate volume replacement, which sometimes requires free water correction with calculation of free water deficits (Li et al, 2013), without increasing the risk of cerebral edema (Odunayo, 2013). Generally, idiogenic osmoles takes approximately 24 hours to develop, so if hypernatremia is chronic (>24 hours) or of unknown duration, sodium concentration should be corrected at lower rates, such as 0.5 mEq/L/hr (Odunayo, 2013). If to rapid correction occurs a delayed pontine myelinolysis can occur resulting in permanent neurological injury which may be fatal.

Due to the abrupt decrease in renal excretion of hydrogen ions, ammonium and phosphorus, patients with AKI usually develop metabolic acidosis (Li et al, 2013). Although the therapy for metabolic acidosis is to treat the underlying cause, in cases where it is not possible further therapy may be required. Acidosis can cause adverse effects if present for over 24 hours, such as decrease in myocardial contractility, cardiac output and blood pressure (BP), shifts in the oxyhemoglobin curve to the right allowing more O_2 to be released (the Bohr Effect) can also occur, altered drug toxicity and activity and decreased binding of norepinephrine to its receptors (Sabatini & Kurtzman, 2009); bicarbonate therapy may be required if the patient's pH is <7.2 or the serum bicarbonate level is less than 14 mEq/L after correcting the fluid deficits (Ross, 2011; Li et al, 2013). Bicarbonate has several side effects, such as intracellular acidosis due to the diffusion of CO_2 across the cell membrane, volume expansion, hypernatremia, hypocalcaemia, in presence of compromised cardiac output congestive heart failure with pulmonary edema may occur, and increases blood lactate and ketone bodies in animals and humans. This therapy has been associated with an increase in mortality, both in humans and experimental animals, and therefore bicarbonate should be reserved to those severely affected (Sabatini & Kurtzman, 2009).

2.4.4 Renal replacement therapy

Renal replacement therapy (RRT) is used when patients have a refractory response to medical therapy. It is not clear the appropriate time to institute dialytic therapy but there are indications to assist the clinician (Figure 8). Dialytic therapy is mainly supportive of renal

function and it is used to provide further time for the patient to recover, by reducing the degree of uraemia and associated anorexia, vomiting, mucosal ulceration and coagulopathy, volume overload and electrolyte inbalance (Langston, 2017). The most common modalities of RRT used in veterinary medicine are peritoneal dialysis (PD) and extracorporeal renal replacement therapy (ERRT), which includes intermittent haemodialysis (IHD) and continuous renal replacement therapy (CRRT) (Li et al, 2013). PD is a time-consuming procedure that its used to remove uremic toxins by diffusion from the peritoneal membrane into the abdominal infused dialysate, which is then drained (Langston, 2017). The osmotic gradient is due the presence of osmotic agents, such as dextrose, in the dialysate that draws fluid across the peritoneum in a process named ultrafiltration. The advantages of this procedure are technical simplicity, excellent cardiovascular tolerance and decreased risk of bleeding (Gabriel et al, 2006). Common complications are catheter occlusion and peritonitis. ERRT removes the uremic toxins by diffusion from the bloodstream that goes through a dialyzer. IHD involves a rapid blood and dialysate flow, which removes efficiently the uremic toxins, allowing the treatment to be done in intervals, although the toxin blood levels can rise between intervals. CRRT involves a dialysate flow rate much slower compared with IHD, but the duration of treatment is longer (Langston, 2017).

RRT is indicated in severe AKI cases in which renal function is suspected to be reversible or partially reversible (Li et al, 2013).

Figure 8 – Indications for RRT (Adapted from Langston, 2017)

Indications for Renal Replacement Therapy
Inadequate urine production
Life-threatening pulmonary edema or fluid overload
Hyperkalemia or other life-threatening electrolyte or acid-base disturbance
Hyperkalemia refractory to medical therapy
Progressive/unremitting azotemia
Diuretic resistant congestive heart failure or severe overhydration in absence of renal disease
Acute poisoning/drug overdose with substance that can be removed by dialysis

Patients characteristics for Renal Replacement Therapy
≥ 2.5 kg
Systolic blood pressure ≥ 80 mmHg
Tractable

2.4.5 Management of uremic complications

2.4.5.1 Systemic hypertension

A common complication seen in approximately 80% of patients with AKI is systemic hypertension and it may be exacerbated by over hydration (Ross, 2011; Li et al, 2013). Monitoring blood pressure every eight to twelve hours in patients with AKI is essential since the probability of hypertension occuring is not related to the severity of the patient's azotaemia (Li et al, 2013). The treatment of hypertension may rely on reducing the rate of IV fluids administered, administration of diuretics, and dialysis to remove excess of fluid, in oliguric or anuric patients (Ross, 2011). Most antihypertensive drugs are only available in oral formulations, and the vomiting associated with AKI often blocks the effects of oral medication and therefore the decision to start an antihypertensive therapy should be based on the severity of the hypertension and the risk of targeting organ damage (Ross, 2011; Li et al, 2013). If antihypertensive medications are required (systolic blood pressure > 180 mmHg) amlodipine (0.18 to 0.3 mg/kg PO q 24 h for cats, 0.2 to 0.4 mg/kg PO q 24 h for dogs) may provide a response within 24 to 48 hours. If the blood pressure does not improve within a few hours, additional administrations can be done in dogs, up to a maximum of 1 mg/kg/day (Langston, 2017).

2.4.5.2 Gastrointestinal complications

Vomiting is one of the most common signs of uraemia in AKI patients and its pathogenesis is multifactorial. Usually it involves stimulation of the chemoreceptor trigger zone by uremic toxins and hypergastronaemia (in dogs) which results in increased hydrochloric acid secretion that leads to inflammation, gastric ulcers and gastritis (Ross, 2011; Li et al, 2013). Cats with CKD appear to have gastric fibrosis and mineralization, instead of gastropathy lesions due to hypergastronaemia. This highlights the importance to control hyperphosphatemia and renal secondary hyperparathyroidism in these patients, since mineralization is caused by calcium metabolism issues rather than hyperacidity. In these patients, gastrointestinal signs, such as vomiting is usually due to uremic toxins interacting with the chemoreceptor trigger zone in the brain (McLeland, Lunn, Duncan, Refsal & Quimby, 2014).

Symptomatic patients benefit from centrally acting antiemetics administration (Figure 9), such as maropitant or metoclopramide, and antacids, such as omeprazole or famotidine (Ross, 2011; Li et al, 2013). However, a study conducted by Tolbert et al, (2017) suggested that famotidine, when used twice a day in dogs, loses efficacy over time and therefore it is recommended caution when using long-term oral administration of famotidine in dogs. In patients with gastric ulcers induced by NSAIDs or exposed to overdose of NSAIDs in general, misoprostol (a prostaglandin analogue) should be administered.

Figure 9 – Drugs for gastrointestinal complications (Adapted from Langston, 2017)

DRUG	INDICATION	DOSAGE—DOGS	DOSAGE—CATS	ADJUSTMENT FOR RENAL FAILURE AND COMMENTS
Famotidine	Decrease acid	0.5-1 mg/kg PO, IM, IV q 12-24 h	0.25-0.5 mg/kg PO, SC q 24 h	Prolong interval or decrease dosage
Omeprazole	Decrease acid	0.5-1 mg/kg PO q 24 h	0.7 mg/kg PO q 24 h	Do not open capsules
Pantoprazole	Decrease acid	0.5-1 mg/kg IV (over 15 min) q 24 h	0.5-1 mg/kg IV (over 15 min) q 24 h	
Metoclopramide	Antiemetic, motility modifier	0.1-0.5 mg/kg PO, SC, IM q 6-8 h, 0.01-0.02 mg/kg/h CRI	0.2-0.4 mg/kg SC q 6-8 h or 0.01-0.02 mg/kg/h CRI	Decrease dosage
Ondansetron	Antiemetic	0.1 mg/kg PO q 12-24 h	0.1 mg/kg PO q 6-8 h, 0.1-0.3 g/kg IV q 6-8 h	
Dolasetron	Antiemetic	0.5 mg/kg PO SC, IV q 24 h	0.5 mg/kg PO, SC, IV q 24 h	
Maropitant	Antiemetic	2 mg/kg PO q 24 h or 1 mg/kg SC q 24 h for 5 days	1 mg/kg PO, SC daily for 5 days	
Mirtazapine	Antiemetic, appetite stimulant	1.1-1.3 mg/kg PO q 24 h	1.88 mg per cat PO q 48 h	
Chlorpromazine	Antiemetic	0.2-0.5 mg/kg IM, SC q 6-8 h	0.2-0.5 mg/kg IM, SC q 8 h	
Prochlorperazine	Antiemetic	0.1-0.5 mg/kg IM, SC q 8-12 h		
Misoprostol	Cytoprotective PGE analogue	1-3 mcg/kg PO q 6-12 h		
Cisapride	Motility modifier	0.1-0.5 mg/kg PO q 8-12 h	2.5-5 mg/cat PO q 8-12 h	

CRI, Continuous rate infusion; *PGE*, prostaglandin.

2.4.5.3 Nutritional management

Animals with AKI are often anorexic and at risk of becoming malnourished due to uraemia, which leads to gastrointestinal ulceration and nausea. Therefore, nutritional support to promote anabolism is recommended until the severity of azotaemia and gastrointestinal complications have been reduced (Ross, 2011; Li et al, 2013). Adverse consequences related with malnutrition are immunosuppression, decreased tissue synthesis and repair, including renal tubular cells, and altered drug metabolism (Ross, 2011). If the patient is not vomiting, enteral supplementation can be administered with feeding tubes (Ross, 2011; Li et al, 2013).

2.5 PROGNOSIS

AKI has been associated with high mortality and morbidity, which is influenced by the underlying aetiology and options available for management (Brown et al, 2015).

The prognosis for dogs and cats with AKI has been reported to be related with the cause, and the mortality reported is approximately 50 per cent. Dogs with AKI may have indicators that imply a poor prognosis, such as severe azotaemia with initial creatinine greater than 884 μmol/l, anaemia below 33 per cent at the time of diagnosis, proteinuria, hypocalcaemia (less than 2.2 mmol/l) and comorbid disorders, such as pancreatitis, sepsis or disseminated intravascular (Ross, 2011; Li et al, 2013); others studies have shown no correlation between creatinine and outcome. Age can be an important factor since studies show that older dogs are at increased risk for AKI and less likely to recover (Brown et al, 2015). Cats with AKI abnormalities such as hypoalbuminaemia and hyperkalaemia at initial diagnosis are more likely to have a decreased survival (Ross, 2011; Li et al, 2013).

A study performed by Harison et al (2012) reported the same that most human studies, which is that higher levels of serum creatinine concentration are associated with worse outcomes.

Recovery from AKI has been associated with a transition from an initial oliguric phase to a polyuric phase, despite this, many patients are presented with nonoliguric AKI. Nonoliguric AKI is more common in nephrotoxic causes rather than ischemic renal injury and therefore it appears to have a better prognosis (Brown et al, 2015).

Brown et al (2015) compared the urine production in dogs with AKI and this association with survival and reported that there was no significant difference in urine production between surviving and nonsurviving dogs at presentation, but the surviving group produced more urine over the study period. Therefore, they concluded that anuria or oliguria persisting beyond 7 days is likely to imply a more negative prognosis than the presence of anuria or oliguria at initial presentation. Also, a degree of hyperkalaemia that increases by 1 mmol/l above 6 mmol/l has been associated with a worsening outcome.

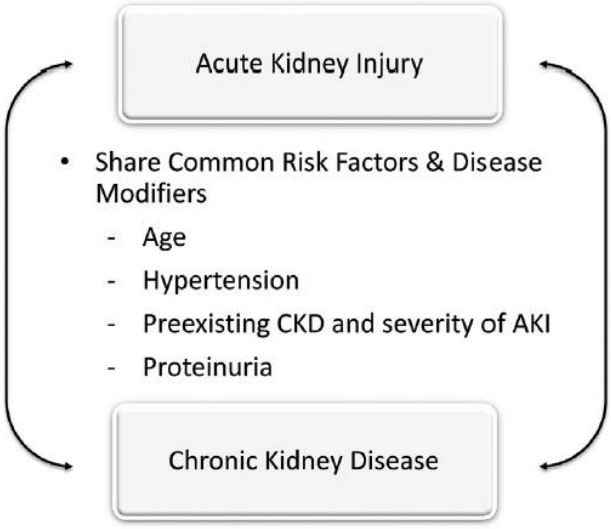
PART III – RENAL BIOMARKERS A LITERATURE REVIEW

3.1 INTRODUCTION

Human studies have suggested that AKI and CKD are two entities closely associated and connected with common risk factors and disease modifiers, as age, hypertension, pre-existing CKD and severity of AKI, and proteinuria, each being a dependent risk factor for the other (Figure 10). This association between the two diseases highlighted the importance of having biomarkers that reflect both functional and structural damage in order to get an early diagnosis and therefore prevent a further disease progression (Yerramilli, Farace, Quinn, & Yerramilli, 2016).

The most accurate assessment of renal function is the measurement of GFR and it is the most sensitive method for early detection of kidney dysfunction, as it is directly proportional to functional renal mass (Cobrin, Blois, Kruth, Abrams-Ogg, & Dewey, 2013; De Loor, Daminet, Smets, Maddens & Meyer, 2013; Kovarikova, 2015; Yerramilli et al, 2016). However, these measurements are costly and time consuming and not suitable to be done in most of the practices, although the RVC in UK uses the iohexol assay from deltaDOT, which is known to provide a highly accurate measure of the GFR (Von Hendy-Willson & Pressler, 2011). New options to detect early AKI have been studied prior to a reduction in GRF.

Figure 10 – Nexus between CKD and AKI (Adapted from Yerramilli et al, 2016)



3.2 DEFINITION OF A BIOMARKER

A biomarker is an indicator of a physiologic or pathological process that has diagnostic and/or prognostic utility (De Loor et al, 2013; Yerramilli et al, 2016).

Biomarkers are usually not specific to an organ, since some proteins markers are secreted by multiple tissues, and therefore it is important to target differences, that are specific to a disease process in order to avoid false diagnoses (Yerramilli et al, 2016).

Ideal biomarkers for detection of kidney disease need to be specific with known reference limits, non-invasive, accurate, and sensitive in order to detect the disease as early as possible. It also should allow the recognition of severity of the disease, be suitable for monitoring, informative about the localization of injury and clinical outcome and prognosis, have a reasonable cost and be rapidly available from a reference laboratory or in practice (cage side) (Cobrin et al, 2013; De Loor et al, 2013; Kovarikova, 2015; Yerramilli et al, 2016).

3.3 MARKERS OF GLOMERULAR DYSFUNCTION

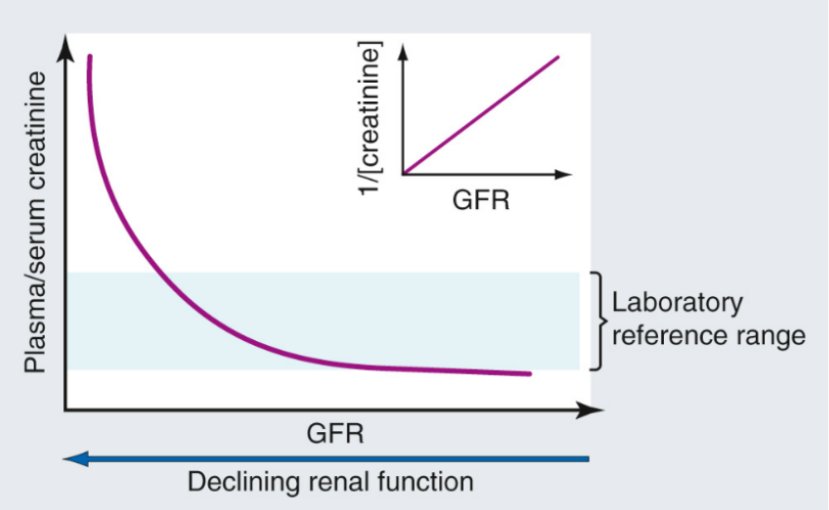
3.3.1 Creatinine

Traditionally, serum creatinine is a standard test for kidney function; although it is insensitive, since increases in serum creatinine usually remain within the reference range (Figure 11), until there is a reduction of 39%-68% in GFR or a reduction in renal mass of 75%. Usually, large changes in GFR early in the disease process result in no to minimal increase in serum creatinine, whereas in end stage disease small changes in GFR result in marked increases in creatinine (Cobrin et al, 2013; Kovarikova, 2015; Yerramilli et al, 2016). Serum creatinine is dependent on factors which are independent of kidney function (Edelstein, 2008). Since creatinine is a product of muscle metabolism, it varies with muscle mass, meaning that in young animals or those with poor muscle mass the kidney function is overestimated, whereas in mature or well-muscled animals it may result in false diagnose of kidney dysfunction (Yerramilli et al, 2016; Syme & Jepson, 2017).

There are several issues related with the analytic measurement of creatinine. For instance, haemolysed samples have an increased release of noncreatinine chromogens that cause an overestimation of creatinine, whereas lipemic and icteric samples can falsely lower the measurement of creatinine. It has been shown that several drugs, such as cephalosporins, aminoglycosides, trimethoprim and phenacemide, cause false increases in creatinine levels (Yerramilli et al, 2016).

Events, that can increase the creatinine value independently of GFR is the ingestion of cooked meat products (Yerramilli et al, 2016). There are also biological processes that can increase creatinine concentrations independently of the kidney function, such as hydration status, changes in tubular secretion and alterations in transport (Cobrin et al, 2013; Yerramilli et al, 2016).

Figure 11 – Relation between creatinine concentrations and GFR (Adapted from Syme & Jepson, 2017)



3.3.2 Symmetric dimethylated arginine

Symmetric dimethylated arginine (SDMA) and asymmetrical dimethylated arginine (ADMA) are two structural isomers, related with a posttranslational modification of the protein arginine in the mitochondria which are then released to the circulation. Approximately 80 % of ADMA is used in enzymatic pathway and therefore it is a poor marker for renal function. However, SDMA is eliminated in the kidneys by renal filtration and excretion, which makes the serum concentration dependent upon changes in GFR (Yerramilli et al, 2016). An advantage comparing to creatinine is that SDMA is not influenced by muscle mass (Dahlem et al, 2017).

Recent studies have shown that a SDMA level above reference range can be found with a correspondent 40% loss of measured GFR (Yerramilli et al, 2016). This implies that SDMA is an earlier marker of kidney dysfunction comparing to creatinine.

Several human studies were done to understand if the serum concentration of SDMA was dependent on other processes or diseases and increased SDMA levels only occurred when AKI was observed. The IRIS recognized SDMA as a biomarker for kidney injury function in dogs and cats, and a clinical chemistry assay, developed by IDEXX, can be used for diagnosis (Yerramilli et al, 2016).

In the study conducted by Dahlem et al (2017) results showed that there is no significant difference in the plasma concentration of SDMA between dogs with AKI and dogs with CKD, which was expected since GFR is decreased in both AKI and CKD, and therefore cannot differentiate between AKI and CKD. Despite this, plasma SDMA concentration is an appropriate biomarker for recognizing AKI and CKD, since dogs with AKI or CKD have a markedly elevated SDMA concentration compared to healthy dogs (Dahlem et al, 2017).

3.3.3 Cystatin C

Cystatin C is a non-glycosylated protein, which inhibits cysteine proteases (Yerramilli et al, 2016; Syme & Jepson, 2017), produced at a constant rate by nucleated cells, and it is filtered through the glomerulus and reabsorbed in the proximal tubules (Cobrin et al, 2013; De Loor et al, 2013; Pressler, 2013; Kovarikova, 2015; Yerramilli et al, 2016). The serum concentration of Cystatin C is determined by GFR, because of its low weight and constant production rate, and therefore it increases when proximal tubular damage occur (Cobrin et al, 2013; De Loor et al, 2013; Pressler, 2013; Kovarikova, 2015). Studies have shown that higher urinary concentrations of Cystatin C can be due to massive proteinuria since it leads to inhibition of Cystatin C tubular reabsorption, and therefore measurements of total proteinuria is required (Kovarikova, 2015).

Limitations to the use of Cystatin C as a marker of GFR are thyroid disorders and glucocorticoid therapy, which may affect cystatin C independently of kidney function (Edelstein, 2008).

3.3.4 Albumin

Albumin is the main protein in plasma. It is produced in the liver, and one of its functions is acting as a carrier protein in order to maintain the oncotic pressure (De Loor et al, 2013; Kovarikova, 2015). Due to its size and the glomerular selective permeability, albumin is not usually present in large quantities in the glomerular filtrate. Since, it is almost completely reabsorbed by tubular epithelial cells, albuminuria frequently represents kidney dysfunction (De Loor et al, 2013; Pressler, 2013), either by glomerular damage, which increases the leakage of albumin or by tubular damage, which decreases the ability of the nephron to degrade the albumin in the glomerular filtrate (Kovarikova, 2015). The standard screening tests to detect proteinuria are urine dipsticks (Pressler, 2013; Kovarikova, 2015).

Albuminuria is not affected by microscopic haematuria, and it is more likely to appear in dogs with pyuria and concurrent haematuria or bacteriuria (Kovarikova, 2015). In critically dogs, microalbuminuria, which is corresponds to a concentration of albumin of > 1mg/dl (Syme & Jepson, 2017), is associated with shorter survival, while in cats, it is associated with the presence of an underlying condition, such as neoplasia, infections, inflammatory or immune-mediated diseases and endocrine disorders (Kovarikova, 2015).

Microalbuminuria can appear in non-renal conditions, and therefore it is not specific for diagnosing renal conditions. Although marked albuminuria is typically related with glomerular disorders (De Loor et al, 2013; Kovarikova, 2015).

3.3.5 Immunoglobulin G

Immunoglobulin G is a high weight protein, which has an important role in humoral response and it is unable to get through an intact glomerular barrier. Therefore, detection of higher concentrations of IgG in urine indicates glomerular injury (Kovarikova, 2015).

3.3.6 C-reactive protein

C-reactive protein (CRP) is an acute phase protein, and therefore its serum concentration increases in a lot of inflammatory conditions. Due to its size, it is not able to go through an intact glomerular barrier, so the presence of CRP in urine is the result of glomerular dysfunction. Studies have shown that for CRP to appear in urine, its serum concentration must be increased and the glomerular barrier must be sufficiently damaged to allow high weight protein filtration (Kovarikova, 2015).

3.4 MARKERS OF TUBULAR INJURY

3.4.1 Urinary enzymes

3.4.1.1 Gamma-glutamyl transpeptidase

Gamma-glutamyl transpeptidase (GGT) is a proximal tubular enzyme (Pressler, 2013; Syme & Jepson, 2017). Studies have shown that GGT is influenced by the urine pH, so in alkaline urine the concentration is higher (Kovarikova, 2015) and that it is also influenced by gender in dogs (Syme & Jepson, 2017).

3.4.1.2 N-acetyl-β-D-glucosaminidase

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme, present in the renal proximal tubule cells (Kovarikova, 2015; Syme & Jepson, 2017). There are two isoenzymes, NAG-A, which is located on the brush border and mainly detected in urine due to exocytosis, and NGA-B which is a intracellular enzyme so it would only appear in urine if the tubular cells are damaged (Cobrin et al, 2013; Kovarikova, 2015; Syme & Jepson, 2017;).

Several studies shown that NAG has some important characteristics that could lead to it having value for use as a biomarker, including no circadian variations in urinary NAG (uNAG) excretion in dogs and cats, no significant difference in uNAG in young and older healthy dogs, no differences in gender in dogs and cats, it is not affected by urine pH in dogs, and its urinary concentration is similar whether it was a free collection or a cystocentesis (Kovarikova, 2015).

3.4.1.3 Alkaline phosphatase

Alkaline phosphatase is an enzyme located in the brush of the proximal tubular cells, and its increase in urine has been associated with proximal tubular damage in dogs. This enzyme can be used to evaluate renal function in several conditions (Kovarikova, 2015).

3.4.2 Low molecular weight proteins

3.4.2.1 Retinol binding protein

Retinol binding protein (RBP) is a low molecular weight protein synthesised in the liver and it circulates in plasma transporting retinol, where 90% of the complex is bound to transthyretin, preventing the passage of the complex through the glomerulus. The free fraction is filtered through the glomeruli and is reabsorbed in the proximal tubules and then catabolised, so increases in urinary RBP may occur in dogs with proximal tubule dysfunction (Cobrin et al, 2013; De Loor et al, 2013; Pressler, 2013; Kovarikova, 2015).

3.4.2.2 Microglobulins

Microglobulins are proteins which are filtered through the glomerulus and reabsorbed by the proximal tubules (Kovarikova, 2015; Syme & Jepson, 2017). An increase of alpha$_1$-microglobulin urine concentrations, occur when reabsorption is reduced, due to a disturbance in tubular function. This microglobulin is stable in different urine pH values and at room temperature (Kovarikova, 2015). Studies showed that urine beta$_2$-microglobulin : creatinine ratio increases prior to azotaemia, being an independent predictor of GFR. However, this microglobulin has a limited utility due to poor thermic stability and instability at acid pH (Syme & Jepson, 2017).

3.4.3 Tubular proteins

3.4.3.1 Clusterin

Clusterin is a glycoprotein. It is expressed by multiple tissues and its part of several physiologic processes, such as sperm maturation, lipid transportation, complement inhibition, tissue remodelling, membrane recycling, and stabilization of stressed proteins, and is an inhibitor of apoptosis (Yerramilli et al, 2016).

Clusterin is expressed in urine at low levels in the healthy kidneys, and those levels increase significantly if an injury occurs. Moreover, studies show that urinary clusterin levels decrease in response to a kidney injury recovery, making it a possible biomarker for kidney damage or disease (Yerramilli et al, 2016).

Contamination of a urine samples with blood can lead to false positives, since this contamination brings nonspecific clusterin isoforms into the sample, and therefore there is not possible to measure the kidney specific clusterin. Contamination, even as smaller, is almost impossible to avoid, since it can happen due to infection, trauma, neoplasia, inflammation and contamination during catheterization and cystocentesis (Yerramilli et al, 2016).

Despite, sampling issues, urinary clusterin can be a sensitive and specific marker for active kidney injury, as long as only kidney specific clusterin can be measured (Yerramilli et al, 2016).

3.4.3.2 Inosine

The energy source of the cell is the ATP, and its levels decrease when energy is used, since it is hydrolysed to ADP, to AMP, and then to adenosine (Yerramilli et al, 2016).

Renal hemodynamic may depend on the adenosine concentrations in the interstitium, since when the interstitial adenosine concentrations increase due to an inhibition of cellular uptake of adenosine and therefore the circulating concentrations decrease, this leads to a decrease in renal blood flow and GFR. Usually the adenosine concentration in the interstitium is low, but during hypoxia and inflammation it increases due to the hydrolysis of ATP and release from injured or apoptotic cells (Yerramilli et al, 2016).

Studies have shown that in dogs the interstitial adenosine is converted into inosine during hypoxic episode. Results suggest that inosine is a sensitive biomarker to injury, and it also responds to injury by restoration of the normal circulating concentrations, in order for the kidney to recover (Yerramilli et al, 2016).

3.4.3.3 Neutrophil gelatinase-associated lipocalin

Neutrophil gelatinase-associated lipocalin (NGAL) is a glycoprotein that binds siderophores, which are iron containing ligands. It was initially purified from neutrophils during infection and inflammation (Hsu, 2014) and it is found in several tissues, such as skin, alveolar and oral mucosa, adipose tissue, and proximal and distal tubules (Pressler, 2013; Yerramilli et al, 2016). In injured organs, including kidney, stomach, colon, liver, trachea and lung, and in neoplasia this protein is induced, being its function to bind extracellular iron, and thereby inhibiting bacterial growth (Pressler, 2013; Kovarikova, 2015; Yerramilli et al, 2016). This protein can bind siderophores from prokaryotes, functioning as a bacteriostatic, and eukaryotes by helping carrying iron across the cellular membranes for cellular proliferation and differentiation (Yerramilli et al, 2016), and it is also involved in the attenuation of apoptosis (Hsu et al, 2014).

In humans, plasma and urine NGAL concentrations are used as a marker of AKI, even in people with underlying CKD, since these concentrations increase with renal damage (Steinbach, Weis, Schweighauser, Francey & Neiger, 2014).

NGAL is one of the earliest biomarkers in ischemic and nephrotoxic animal models of AKI (Lee et al, 2012; Segev, Palm, Leroy, Cowgill & Westropp 2013).

Studies have shown that in dogs NGAL concentration increases earlier that serum creatinine in AKI (Cobrin et al, 2013; Yerramilli et al, 2016). It increases dramatically when the renal insult occurs, and then gradually decreases with time (Hsu et al, 2014), allowing it to be measure only until a few hours after the injury occurs.

Lee et al (2012) concluded that an increase in urinary NGAL concentration can predict kidney injury in dogs after surgery, since in comparison to serum creatinine for the diagnosis of AKI at 48h post-surgery, urine NGAL is able to diagnose AKI 12h after surgery in dogs.

A study conducted by Steinbach et al (2014) has shown that dogs with renal azotaemia had higher plasma and urine NGAL concentrations compared with healthy dogs. Other results, from this study were that plasma NGAL seems to be less sensitive compared with urinary NGAL. The authors explain that in this study the increase in plasma NGAL in dogs with AKI can be due to 2 mechanisms, the up-regulation of inflammatory genes, one of them which codes for NGAL, and the reduced filtration rate which leads to reduced clearance of NGAL and therefore systemic accumulation.

NGAL circulating concentrations may be influenced by coexisting conditions, such as CKD, chronic hypertension, systemic infections, inflammation, anaemia, and hypoxia. Urinary NGAL concentrations seem to be related with serum creatinine concentrations, GFR and proteinuria (Yerramilli et al, 2016).

In patients with sepsis, NGAL expression increases, not only in the kidney, but also in leukocytes and liver, and therefore NGAL urine and blood concentrations are increased, in absence of AKI (Cortellini, Pelligand, Syme, Chang & Adamantos, 2015). The results of a study conducted by Cortellini et al (2015) confirmed that sNGAL is increased in dogs with sepsis. The inability of NGAL to distinguish between the presence of AKI and systemic inflammation in septic dogs, suggest that this is a poor marker of AKI in these patients (Cortellini et al, 2015).

Further studies need to be done to assess if NGAL can be used as a predictor of AKI in cases of toxicosis by nephrotoxins, such as dogs that have ingested grapes or raisins, or cats that have been exposed to lilies, to determine if the patient needs to stay at the ICU after being exposed to a renal toxin or if the patient is unlikely to develop AKI.

4.1 AKI IN DOGS AFTER INGESTION OF GRAPES OR RAISINS

Fruits of the *Vitis* genus, such as grapes, raisins, sultanas and currants may be toxic for dogs if ingested, causing AKI, but the toxic component has not been identified and the mechanism of toxicosis is still unknown (Eubig et al, 2005; Morrow, Valli, Volmer & Eubig, 2005; Hovda et al, 2016). AKI does not always occur after the ingestion of grapes or raisins and therefore there is no way of knowing, after the ingestion if that dog is going to develop AKI or not (Hovda et al, 2016).

Reich et al, (2016) evaluated the clinical course and outcome in 130 dogs with known grape and raisin ingestion, and results from this study showed that with treatment (decontamination and fluid therapy) the incidence of AKI was 8.5%. Whereas recovery has been reported to occur in only 50% of patients with grapes and raisins toxicity (Eubig et al, 2005; Morrow et al, 2005).

In the study conducted by Eubig et al (2005), the results showed that, those dogs outcome was not influenced by the number of raisins ingested, yet, usually, toxic agents reveal a dose-response relation, in which a changing grade of response indicates that a changing amount of a toxic agent is administered. The authors explained these results, considering two possibilities; one is that the toxic component may be intrinsic and present in different amounts, depending on the environment or plants genetics; the other is that the toxic component may be extrinsic, and therefore not always present. There may also occur different responses among individual dogs, meaning some dogs may be more sensitive to develop AKI than others (Eubig et al, 2005), and therefore decontamination and treatment is recommended for all dogs exposed to these toxins.

There have been proposed several mechanisms that could explain this nephrotoxicity, including presence of ochratoxins, a mycotoxin; pesticides, although the concentrations of pesticides on the fruits decline fast due to evaporation and degradation (Eubig et al, 2005); or components of the fruit that cannot be metabolized, although seedless or seeded grapes have all been reported to be nephrotoxic. The metabolism and excretion of the toxicant is also unknown (Hovda et al, 2016).

The digestion of raisins and grapes is a slow process, and they may be found intact in vomitus or faeces, hours after exposure (Hovda et al, 2016).

The clinical signs occur in 50% of dogs or less that ingest grapes or raisins. Signs include gastrointestinal signs (vomiting, diarrhoea), abdominal pain and anorexia; renal signs, with presence of acute renal tubular necrosis which leads to AKI; hepatobiliary signs, with mild

elevations in liver enzymes; endocrine/metabolic signs such as hypercalcemia and hyperphosphatemia, and metabolic acidosis due to the presence of uremic acids; and rarely neuromuscular signs, including weakness and ataxia. Due to varied signs that could appear with the ingestions of grapes, other differential diagnosis that could explain these signs should be considered, since underlying conditions may be present (Hovda et al, 2016).

Acute exposures are more likely to have a normal physical exam. The initial and most common clinical sign is vomiting, which often occurs between 2 and 24 hours post ingestion, and it is usually followed by anorexia, lethargy, dehydration, and diarrhoea within the next 12 to 24 hours and is associated with the development of AKI (Hovda et al, 2016).

Therapeutics of patients that ingested grapes or raisins should include detoxification, by inducing emesis, up to 6 hours after ingestion due to the slow digestion of these fruits, and administration of activated charcoal, especially if ingested grapes or raisins are not recovered in the vomitus (Eubig et al, 2005). Repeated doses of activated charcoal are not recommended (Hovda et al, 2016). Detoxification should be followed by intravenous fluid therapy for at least 48 hours, since it allows the preservation of renal blood flow, decreases tubular obstruction from necrosis, and decreases the reabsorption of the nephrotoxic agent due to the increased blood flow (Eubig et al, 2005). The blood work should be monitored for 72 hours post ingestion. If symptoms consistent with AKI occur, supportive care and monitoring previously discussed should be instigated. In dogs that continue to vomit, gastric protectants and antiemetics should be administered, such as maropitant, famotidine or omeprazole, to reduce vomiting and the severity of uremic gastritis. These patients do not require a specific diet, but patients that develop AKI after ingesting grapes could benefit from a low-protein, low-phosphorus diet, until renal function becomes normal (Hovda et al, 2016).

The prognosis for acute ingestion of grapes or raisins is good, if appropriate decontamination and fluid therapy is done. For patients that develop AKI, the prognosis may be poor, and there is the possibility for long term renal insufficiency (Hovda et al, 2016). CRRT may be required in severe cases.

4.2 AKI IN CATS AFTER EXPOSURE TO LILIES

Ingestion of lilies from the genera *Lilium* and *Hemerocallis* is known to cause AKI in cats (Bennett & Reineke, 2013; Hovda et al, 2016), by causing renal tubular necrosis (Hovda et al, 2016). Cats are the only species in which acute renal failure has been reported after exposure to lilies (Slater & Gwaltney-Brant, 2011). Lilies are usually sold as potted plants or floral arrangements for indoor use, but are also planted outdoors in gardens (Rumbeiha, Francis, Fitzgerald, Nair, Holan, Bugyei & Simmons, 2004).

The ingestion of both leaves and flowers causes toxicosis, with the kidney being the target organ (Hovda et al, 2016). Rumbeiha et al (2004) concluded that the toxic compounds are present in the aqueous extract of leaves and flowers, although, the toxin has not yet been identified, the water-soluble extract from the flowers is more potent than the leaf extract.

The clinical signs of lily toxicosis, usually develop within 6 to 12 hours of exposure and include gastrointestinal signs, such as vomiting, ptyalism, anorexia, and diarrhoea; renal signs, associated with AKI, such as polydipsia, polyuria, oliguria, anuria, renomegaly, abdominal or renal pain, and oral ulceration; and nonspecific signs as dehydration, hypothermia or fever, lethargy, depression, ventricular premature contractions, and death. Other signs that have also been reported include neurological signs, such as depression, ataxia, head pressing, tremors, and seizures; and pancreatitis, despite rare (Bennett & Reinike, 2013; Hovda et al, 2016). Similar to vitis spp. ingestion due to the several signs that could appear with exposure to lilies, other differential diagnosis that could explain these signs should be considered, since underlying conditions may be present (Hovda et al, 2016). It has been reported that deaths occur, due to acute renal injury 3 to 6 days after exposure (Slater & Gwaltney-Brant, 2011; Rumbeiha et al, 2004) and some deaths have occurred after ingestion of only a small piece of the plant.

The treatment of lily toxicosis consists of early decontamination, prevention of renal injury, and maintenance of fluid, electrolyte, and acid-base balance. Baseline serum chemistries, such as BUN, creatinine, and electrolytes should be done on admission and repeated every day until they plateau or reach normal/baseline values. Decontamination includes bathing cats, which have been contaminated with pollen; inducing emesis with dexmedetomidine, since it is a more effective emetic in cats, if they are presented within 1 to 2 hours of exposure; and administrating one dose of activated charcoal with a cathartic, to bind the toxins in the gastrointestinal tract (Rumbeiha et al, 2004; Hovda et al, 2016;). Prevention of renal injury includes early and aggressive diueresis, within the first 18 hours after exposure (Slater et al, 2011; Bennett et al, 2013) and it should be accompanied with daily serum chemistries and monitored of the urine output (Hovda et al, 2016). The IV fluid therapy should be maintained

at 4-6 ml/kg/hr for 48 hours and then decreased depending on the chemistry values. In well hydrated cats that are oliguric, diuresis can be attempted with furosemide bolus and or CRI or mannitol bolus if patient is not anuric. Gastrointestinal protectants may be used if indicated, such as famotidine or omeprazole. In anuric cats peritoneal or renal dialysis may be useful (Hovda et al, 2016).

The prognosis of lilies toxicosis is good, if cats are aggressively treated within the first 18 hours of exposure. Treatment done after 18 hours from the exposure or longer, usually results in AKI. Once oliguria or anuria develops, the prognosis becomes poor. The mortality rate reported is as high as 100%, if renal failure occurs due to a delay in treatment (Hovda et al, 2016).

PART V – EXPERIMENTAL STUDY

5.1 INTRODUCTION AND OBJECTIVES

Kidney diseases commonly affect dogs and cats (Cobrin et al, 2013; Kovarikova, 2016). Slight changes in kidney function do not result in organ failure but play an important role on the impact of the condition, including morbidity and mortality (Yacoub et al, 2016). In companion animals, severe AKI has a poor prognosis with mortality rates up to 50 and 60%. This is in part due to insensitive diagnostic tests which delay the detection of this condition, the subtlety of early signs, and the rapid progression of the injury associated with nephrotoxins (Cobrin et al, 2013). In humans, the incidence of AKI varies, occurring from 4 to 20% in hospitalized patients and from 35 to 70% in critically ill patients in ICU (Endre, Pickering & Walker, 2011), with a mortality rate approximately 45 to 64% (Cobrin et al, 2013).

The emphasis is now the necessity for early recognition of AKI, in order to be able to use more effective therapeutic interventions, recognise developing injury early and have more positive outcomes. This led IRIS to adopt a grading scheme to categorize and stratify the severity of AKI (Segev et al, 2013). Although the adoption of the new criteria will allow an earlier recognition of renal dysfunction, and therefore, hopefully a more effective treatment (Mugford et al, 2013), the criteria has not yet been validated for use in general patient population. One of this study proposes was to relate the detection of AKI using the IRIS criteria in small animals exposed to a nephrotoxin compared with the detection of AKI using the creatinine reference range alone.

Hayes et al (2010) developed a scoring system for estimation of illness severity in hospitalized dogs, the Acute Patient Physiologic and Laboratory Evaluation (APPLE) score (appendix IV), which reflects the severity of derangement of normal physiology identified by abnormalities in clinical and laboratory variables, and correlates with likelihood of survival to hospital discharge. The 10-variable score ($APPLE_{full}$), which considers as variables creatinine (µmol/L), wbc ($x10^9$/L), albumin (g/L), SpO_2 (%), total bilirubin (µmol/L), mentation score, respiratory rate (bpm), age (years), fluid score and lactate (mmol/L), was adapted to a 5-variable model ($APPLE_{fast}$), which considers glucose (mmol/l), albumin (g/l), lactate (mmol/l), platelet count and mentation score, that can be used when clinical information or available time is more limited. Hayes et al (2011) also developed and validated a score to stratify illness severity in hospitalized cats (appendix V). Both the 8-variable score, which considers as variables mentation score, temperature (ºC), MAP (mmHg), lactate (mg/dl), PCV (%), urea (mg/dl), chloride (mEq/l) and body cavity fluid, and the fast 5-variable score, which

considers mentation score, temperature (ºC), MAP (mmHg), lactate (mg/dl) and PCV (%), are based on variables that are inexpensive to measure and readily available. The study design also included the collection of data for the $APPLE_{fast}$ score which was present in the form (appendix I).

In both human and veterinary medicine, there is a need for more sensitive and specific markers for early identification of AKI. There is a growing interest in the discovery of biomarkers that could help diagnose, classify and grade the severity of AKI. The initial design of this study intended to compare the detection of AKI using the IRIS criteria to increase in NGAL, and to also investigate the early detection of AKI using the IRIS criteria and a urinary biomarker NGAL as compared to the creatinine reference range in the same groups. Unfortunately, due to costs for preserving the samples for batch analysis (in -70ºC environment), this was not possible to do without further funding.

5.2 MATERIAL AND METHODS

5.2.1 Study Population

The study population were client-owned dogs and cats presented at Village Vet Hampstead / Vet24, a 1st opinion hospital practice (RCVS Recognised), with history of exposure to a nephrotoxin. This included, dogs with probable ingestion of grapes or/and raisins, and cats with probable exposure to Lilies. The study was conducted over a three-month period, from November 2016 to January 2017, and a total of 18 patients were recruited (7 dogs and 11 cats). For the statistical analysis patients were divided into two groups, according with species, and variables were compared within each group.

5.2.2 Selection criteria

To enrol the study, the patients had to be of canine or feline species; be presented with an history of confirmed or probable exposure to a renal toxin; have a serum creatinine measurement at admission, and 24 hrs, 48 hrs and 72 hrs after admission; and be hospitalised for 3 days with continuous IV fluid therapy. Exclusion criteria included any patient with an history of a chronic disease and patients with incomplete data collection.

In all samples, creatinine values were measured, at the hospital, on the same chemistry analyser (VETtest 8008, IDEXX), which eliminates intermachine variability. Recent chemistry analysers have a small coefficient of variation when measuring creatinine, and therefore it is unlikely that increases in creatinine occur between samples, when the same analyser is used (Thoen & Kerl, 2011).

The creatinine reference range used was 71 – 212 µmol/L (0,8 – 2,4 mg/dl) in cats and 44 – 159 µmol/L (0,5 – 1,8 mg/dl) in dogs, being considered the presence of AKI when values of serum creatinine were above the high limit of the interval, whereas the IRIS criteria defines AKI as an increase of 26,4 µmol/L (0,3 mg/dl) in creatinine from the baseline value (Brown, 2016). Both criteria were evaluated on each patient for a period of 72 hrs.

5.2.3 Fate of enrolled patients

All patients were managed by their respective clinicians, according to the manage protocols used, as usually, in the practice. The protocol used was designed by a Diplomat of the American College of Veterinary Emergency and Critical Care.

For this study, the blood samples were collected by jugular venipucture or from an intravenous catheter, depended on patient and attended clinician preference, at admission, 24 hours, 48 hours and 72 hours after admission. All samples were collected according to the practice protocol to manage these patients, so no additional blood samples were taken for this study.

5.2.4 Statistical treatment of data

The data documented in the form (appendix I), which was designed for this study, was organized in the program Microsoft Office Excel 2016 and the descriptive and inferential statistical analysis was performed with IBM SPSS Statistics 24. Normality of the data was assessed with the Shapiro Wilk normality test, and variables were considered normally distributed if $p > 0,05$. In the feline group, the T-student test was used to evaluate the relation between the IRIS criteria and the creatinine reference range with variables, such as age, creatinine at time of presentation and weight. This test was performed assuming a 95% confidence interval and with a p value of $<0,05$ being considered significant. To evaluate the relation between the two methods, the chi-square test was used. The McNemar test was performed to evaluate if there was a significantly difference regarding the sensitivity of these methods. To do the power calculation of this study, a different statistic program was used, the program R version 3.2.3.

5.3 RESULTS

5.3.1 Feline group characteristics

The sample included 11 cats (*n* = 11), all hospitalized due to confirmed or probable exposure to a renal toxin, in this case exposure to lilies.

Regarding patient's gender, the study included 6 females and 5 male cats, with ages between 5 months and 12 years (mean (years) ± standard deviation = 4,74 ± 3,84) (chart 2) and weight between 2 kg and 7,4 kg (mean (kg) ± standard deviation = 4,49 ± 1,63) (chart 3).

Chart 2 – Boxplot distribution of feline patient's age

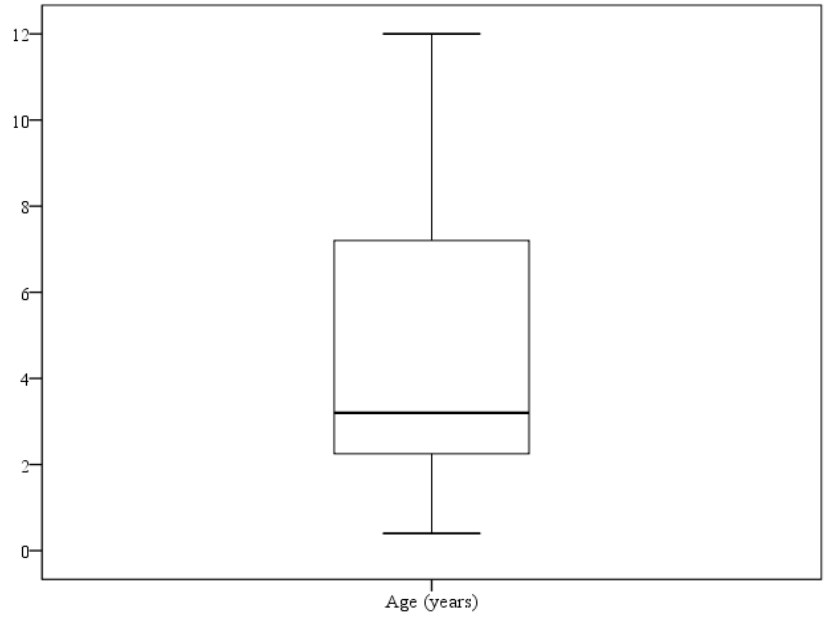
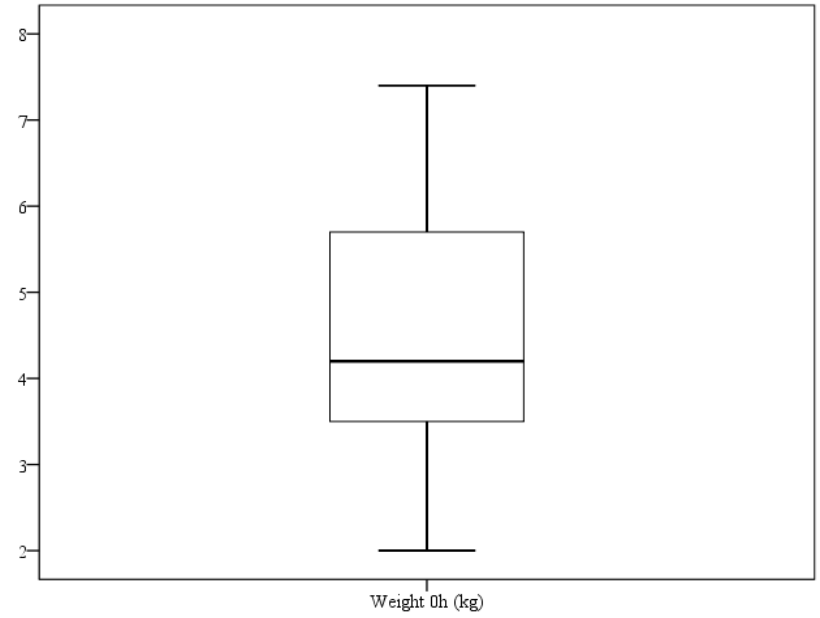
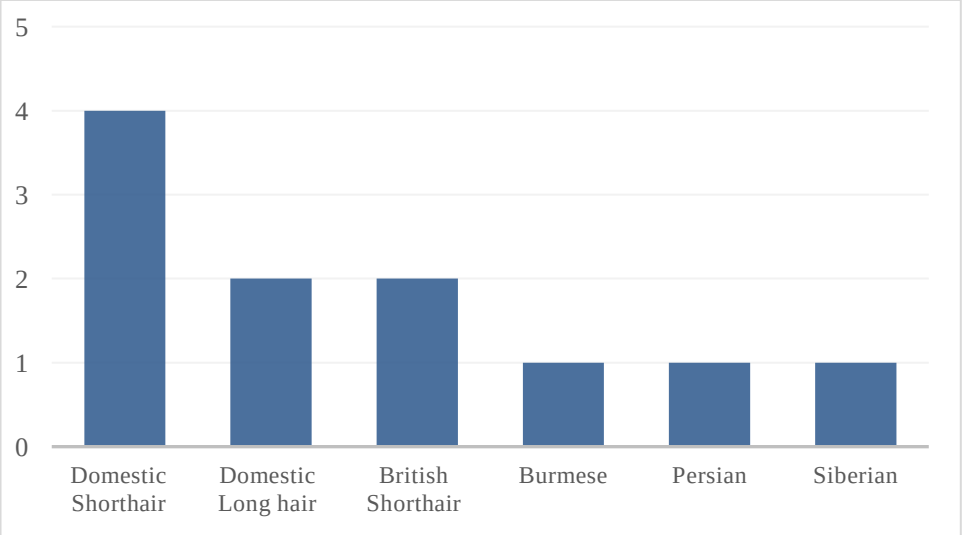


Chart 3 – Boxplot distribution of feline patient's weight



Patient breeds participating in the study included Domestic Shorthair (4 animals), Domestic Long hair and British Shorthair (2 animals each), and Burmese, Persian and Siberian with one animal (chart 4).

Chart 4 – Feline breeds included in the study



In all patients the creatinine range and the IRIS AKI criteria were used to define the presence of AKI. The IRIS criteria define AKI with an increase of 26,4 µmol/L (0,3 mg/dl) in creatinine from the baseline value (Brown, 2016) (Figure 12), and therefore this difference was evaluated in all patients (Table 3).

Figure 12 – IRIS AKI grading criteria (Adapted from Brown, 2016)

AKI Grade	Blood Creatinine	Clinical Description
Grade I	<1.6 mg/dl (<140 µmol/l)	Nonazotemic AKI: a. Documented AKI: (historical, clinical, laboratory, or imaging evidence of AKI, clinical oliguria/anuria, volume responsiveness‡) and/or b. Progressive nonazotemic increase in blood creatinine: ≥ 0.3 mg/dl (≥ 26.4 µmol/l) within 48 h c. Measured oliguria (<1 ml/kg/h)# or anuria over 6 h
Grade II	1.7 – 2.5 mg/dl (141 – 220 µmol/l)	Mild AKI: a. Documented AKI and static or progressive azotemia b. Progressive azotemic: increase in blood creatinine; ≥ 0.3 mg/dl ≥ 26.4 µmol/l) within 48 h),or volume responsiveness‡ c. Measured oliguria (<1 ml/kg/h)# or anuria over 6 h
Grade III	2.6 – 5.0 mg/dl (221 – 439µmol/l)	Moderate to Severe AKI: a. Documented AKI and increasing severities of azotemia and functional renal failure
Grade IV	5.1 – 10.0 mg/dl (440 – 880 µmol/l)	
Grade V	>10.0 mg/dl (>880 µmol/l)	

(‡Volume responsive is an increase in urine production to >1 ml/kg/h over 6 h; and/or decrease in serum creatinine to baseline over 48 h)

Table 3 – Creatinine values during hospitalization

	t = 0 hrs	t = 24 hrs	t = 48 hrs	t = 72 hrs
A	121	133	129	120
B	166	124	135	123
C	66	69	60	68
D	77	70	65	96
E	149	175	**194**	**246**
F	189	164	147	166
G	168	161	159	155
H	133	122	120	110
I	187	129	139	166
J	203	121	177	172
K	204	197	**216**	**240**

The patients were divided into two groups, the AKI group and the NAKI group. When considering the IRIS criteria, the AKI group was composed of two patients (n = 2) and the NAKI group was composed of nine patients (n = 9) (Chart 5). When the creatinine reference range was considered, the AKI group also had 2 patients (n = 2), and the NAKI group had 9 patients (n = 9) (Chart 5). Both IRIS criteria and the creatinine reference range detected AKI in the same patients. The T-student test suggested that relation between both IRIS criteria and the creatinine reference range and variables such as age, creatinine at time of presentation and weight were statistically insignificant. The characteristics of these groups and p values are summarized in table 4.

Chart 5 - Frequency of AKI / NAKI detected by Creatinine range and IRIS criteria

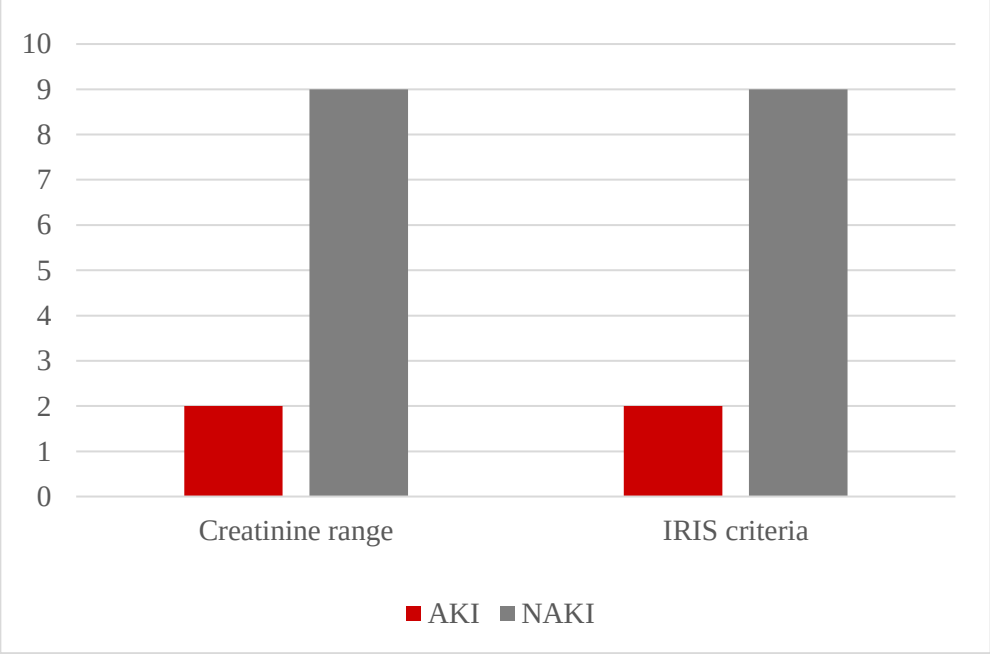


Table 4 – Characteristics of AKI and NAKI groups

	AKI (*n* = 2)	NAKI (*n* = 9)	*p* value
Age (mean (years) ± standard deviation)	6,7 ± 1,70	3,92 ±3,86	0,191
Creatinine oh (mean (µmol/L) ± standard deviation)	176,50 ± 38,89	145,56 ± 49,52	0,445
Weight oh (mean (kg) ± standard deviation)	4,35 ± 0,21	4,26 ± 1,77	0,880

The IRIS criteria and the creatinine reference range were compared, by the chi-square test, with a *p* value of 0,018 (being statistically significant a p value < 0,05), which showed that there is an association between the two methods.

To evaluate sensitivity between these criteria, the McNemar test was performed and a *p* value = 1,00 (being statistically significant a *p* value < 0,05), showed that the two methods are not significantly different with respect to sensitivity.

The power calculation for this test was approximately 16%. To improve the power calculation of this study to 80%, a sample of 68 cats would be needed, being each group composed by 34 cats. To improve the power calculation to 95%, each group would need 56 cats, with 112 cats in total.

5.3.2 Canine group characteristics

The sample included 7 dogs (*n* = 7), all hospitalized due to confirmed or probable exposure to raisins or grapes.

Regarding patient's gender, the study included 4 females and 3 male dogs, with ages between 1 year and 4 years and 11 months (mean (years) ± standard deviation = 2,60 ± 1,69) (chart 6) and weight between 9 kg and 27,2 kg (mean (kg) ± standard deviation = 20,27 ± 6,62) (chart 7).

Chart 6 – Boxplot distribution of canine patient's age

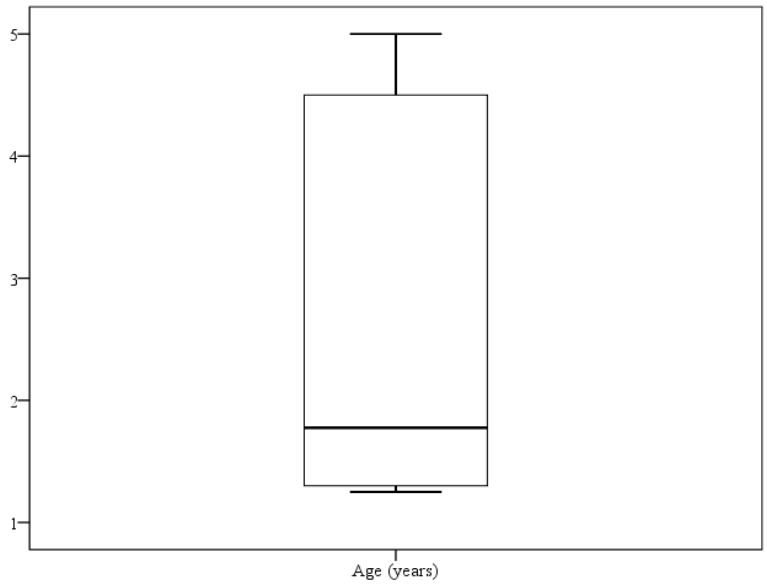
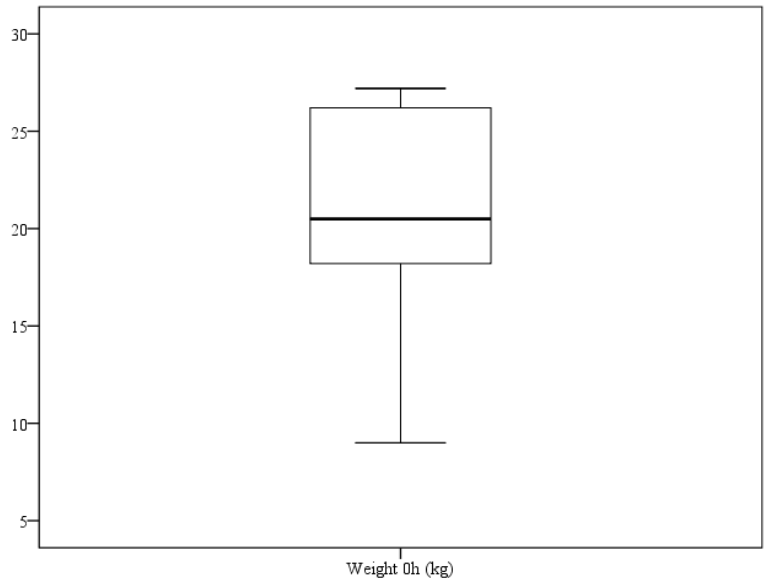
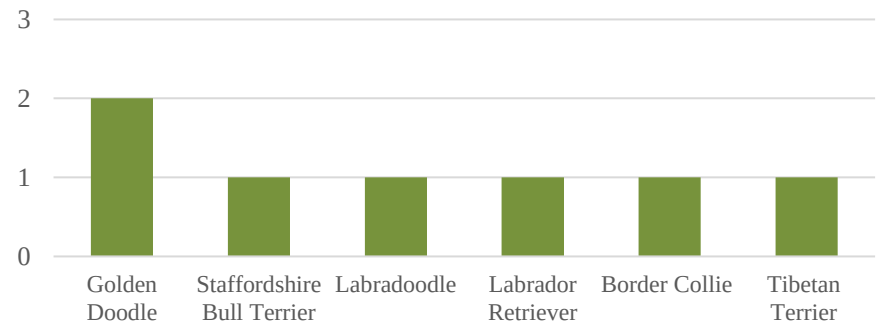


Chart 7 – Boxplot distribution of canine patient's weight



Patient breeds participating in the study included Golden Doodle (2 animals), Staffordshire Bull Terrier, Labradoodle, Labrador Retriever, Border Collie and Tibetan Terrier (1 animal each) (chart 8).

Chart 8 – Canine breeds included in the study



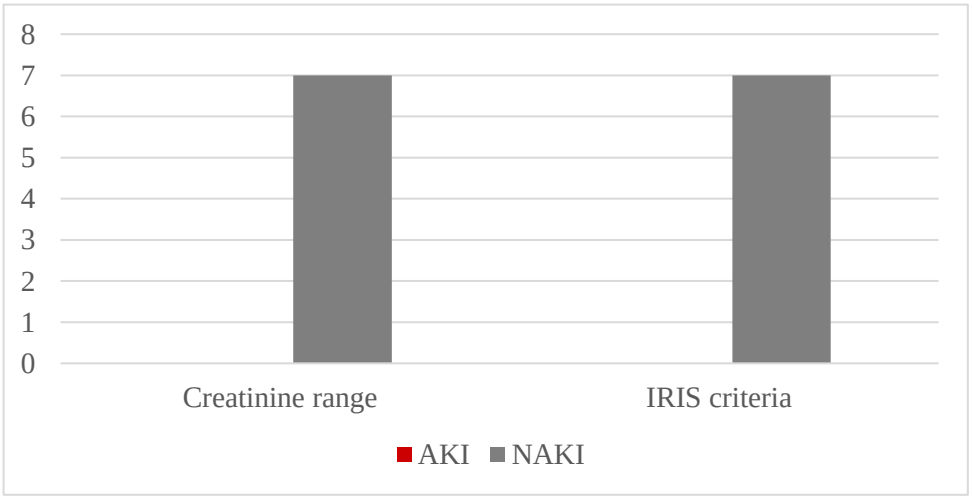
In all patients the creatinine range and the IRIS criteria were used to define the presence of AKI. The IRIS criteria define AKI with an increase of 26,4 µmol/L (0,3 mg/dl) in creatinine from the baseline value (Brown, 2016), and therefore this difference was evaluated in all patients (Table 5). In canine patients none of the methods detected AKI (Chart 9).

Table 5 – Differences in creatinine values during hospitalization

	t = 0 hrs	t = 24 hrs	t = 48 hrs	t = 72 hrs
A	92	109	83	95
B	119	100	97	105
C	106	91	101	106
D	117	94	98	79
E	108	-	116	111
F	120	110	107	102
G	91	86	83	89

The patients were supposed to be divided into two groups, the AKI group and the NAKI group. But none of the criteria detected patients with AKI, so all 7 patients belonged to the NAKI group, independent of the method considered (Chart 9).

Chart 9 - Frequency of AKI / NAKI detected by Creatinine range and IRIS criteria



5.4 DISCUSSION

This study was composed of two samples, one with a total of 11 feline patients and the other with 7 canine patients, each represented by an approximately similar number of patients from both genders and various breeds.

The results of the current study demonstrated that in the feline patients, both creatinine reference range and IRIS criteria identified AKI in the same patients. When these criteria were compared with variables, such as age (p = 0,191), creatinine at time of presentation (p = 0,445) and weight (p = 0,880), the results showed no statistically significant relation between them, being statistically significant a p value < 0,05.

Serum creatinine is a standard test for kidney function; although it is insensitive, since increases in serum creatinine usually remain within the reference range, until there is a reduction of 39%-68% in GFR or a reduction in renal mass of 75% (Cobrin et al, 2013; Kovarikova, 2015; Yerramilli et al, 2016).

This study purpose was to relate the detection of AKI using the IRIS criteria in small animals exposed to a nephrotoxin compared with the detection of AKI using the creatinine reference range alone, since the IRIS criteria has not yet been validated for use in general patient population. It would be expected that the IRIS criteria would allow an earlier recognition of renal dysfunction (Mugford et al, 2013), thereby the hypothesis that the IRIS criteria is more sensitive in detecting AKI compared with creatinine reference range alone, was tested. However, this study results did not support this hypothesis. Since results in the sample do not reflect reality in the population, two types of error may occur. A type I error (false-positive) which occurs if the null hypothesis is rejected but it is true in the population; or a type II error (false-negative), which occurs when the null hypothesis should be rejected but it is not. The likelihood for these errors can be reduced by increasing the sample size, since larger sample sizes are less likely to differ from the population (Banerjee, Chitnis, Jadhay, Bhawalkar & Chaudhury, 2009).

This study was underpowered, with a power calculation of approximately 16%. A power calculation this low does not allow any statistically significant results, and a type I or type II error may occur. When sensitivity between these criteria was evaluated, a p = 1,00 (being statistically significant a p value < 0,05), showed that the two methods were not significantly different with respect to sensitivity. This result was due the fact that both criteria detected the same number of feline patients with AKI, probably because of the small number of patients that composed the sample.

The results of the canine sample showed that any of these criteria detected dogs with AKI. Incidence of AKI in dogs with raisin toxicity with treatment is only 8.5% (Reich et al, 2016).

Whereas recovery has been reported to occur in only 50% of patients with grapes and raisins toxicity (Eubig et al, 2005; Morrow et al, 2005). Since all patients were managed with fluids and monitoring, it would only be expected AKI in 8.5% of the patients, which means that < 1 in every 10 patients would have AKI. Therefore, in this study due to the small number of patients in the canine sample, <10, and since all patients were managed with fluids and monitoring, it was expected to have < 1 patient with AKI, which corresponded to what was observed in this study. As explained before, this study was underpowered, and therefore more patients would be needed to be able to compare both criteria in respect to sensitivity.

A study conducted by Thoen and Kerl (2011) showed that dogs whose creatinine increased > 26,5 µmol/L were more likely to die (54,2% compared to 15,7%) and even dogs whose creatinine only increased marginally, often still within the reference range, were more likely to die (57,9%). In this study, the mortality rate was 0% and the discharge was 100% in both samples.

The $APPLE_{fast}$ scoring was not possible to do because there was not enough data collected for these scores, due to the busy times some patients arrived at the hospital, and therefore the sampling conditions and illness severity were not standardized, making it impossible to say that all patients within the groups were as ill.

During the 3 days of hospitalisation, patients were weighed at presentation and every 12hours, allowing a more accurate fluid therapy. The differences between each day weights were considered, since increases by 10% or more could indicate that fluid was being withheld, and this would mean a smaller urinary output than expected and an adjustment in fluid therapy (Appendix II and III).

This study had a number of limitations. First, the small sample size has rendered this study underpowered to document potential differences between the creatinine reference range and the IRIS criteria with respect to sensitivity for detection of AKI. In feline sample, the creatinine reference range and the IRIS criteria detected the same two cats with AKI and the two methods were not significantly different with respect to sensitivity, which may have been due to the small sample; whereas the canine sample was composed by < 10 patients, who were managed with fluids and monitoring, and since in dogs with raisin toxicity that receive treatment only 8% develop AKI, it was expected that any patient had AKI. Second, there was not enough data collected to apply the $APPLE_{fast}$ score, due to the busy dynamics of the practice, and therefore the illness severity and sampling conditions were not standardized, so no conclusions were taken regarding illness severity in the patients that participated in this study. Third, the costs for preserving the samples for batch analysis (in -70ºC environment), made it impossible to investigate the early detection of AKI and predict outcomes by using a

urinary biomarker as NGAL with the IRIS criteria and compared it to the creatinine reference range within the same groups. Therefore, this is a pilot study and additional studies involving a larger sample size and funding are required, not only to evaluate the sensitivity of IRIS AKI criteria comparing to the creatinine reference range, but also to evaluate how sensitive and specific urinary biomarkers as NGAL can be in early diagnosing, classifying and grading the severity of AKI and predicting outcomes with ideally illness severity scoring.

5.5 CONCLUSION

The information available for studies on sensitivity of IRIS AKI criteria compared with creatinine reference range alone is still very limited, and this criteria has not yet been validated for use in general patient population. Therefore, a lot of clinics still use the creatinine reference range to diagnose AKI.

In both human and veterinary medicine, there is a need for more sensitive and specific markers for early identification of AKI. There is a growing interest in the discovery of biomarkers that could help diagnose, classify and grade the severity of AKI, but there is still a need for further studies in this field.

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APPENDIX I – FORM FOR DATA COLLECTION

Form for patients presented with possible renal toxin exposure

Patient Information (Print Label)

Age: ___ years ___ months

Reason for presentation: _____
Time from exposure (hours): _____ hours (Estimate/Exact) (Please, select an option)
PU/PD: YES/NO (Please, select an option)

Physical Exam:

$APPLE_{fast}$

Mucous membranes	
CRT (seconds)	
Heart Rate	
Respiratory Rate	
Temperature[2] (ºC)	

NIBP[2] (Doppler)	
Glucose[1] (Glucometer)	
Lactate[1,2] (Lactometer)	
PCV[2] (%)	
Albumin[1] (VETtest)	
Platelet count[1] (Procyte Dx)	

Mentation Score[1,2]: (Please, select an option)

0. Normal
1. Able to stand unassisted, responsive but dull
2. Can stand only when assisted, responsive but dull
3. Unable to stand, responsive
4. Unable to stand, unresponsive

Creatinine (blood sample)

Admission	24 hrs	48 hrs	72 hrs

(If not from jugular, please, specify _____)

Did Creatinine go above reference number?

Admission YES/NO	24 hrs YES/NO	48 hrs YES/NO	72 hrs YES/NO

Urine Specific Gravity (free catch) _____

Body weight Kg (for calculating the urinary output)

Admission	12 hrs	24 hrs	36 hrs	48hrs	60 hrs	72 hrs

Fluid Rate	Admission	24 hrs	48 hrs	72 hrs
	Bolus ___ml/___min ___ml/___min ___ml/___min	Bolus ___ml/___min ___ml/___min ___ml/___min	Bolus ___ml/___min ___ml/___min ___ml/___min	Bolus ___ml/___min ___ml/___min ___ml/___min

Acute Kidney Injury / Non Acute Kidney Injury (Please, select one option)

Discharged / Non-discharged (Please, select one option)

If Non-discharged: Died / Euthanasia (Please, select one option)

If Euthanized: Financial constraints / Current severity of illness (Please, select one option)

APPLE$_{fast}$ Score: It will allow us to say that both groups of patients were as sick, when we compare the study results.

[1]Canine APPLE$_{fast}$ Score
[2]Feline APPLE$_{fast}$ Score

Thank you very much for all your help!!! ☺

Any questions, please, call Adam or ask Ana

APPENDIX II – DATA COLLECTED IN THE FELINE GROUP

	Age (years)	Sex	Reason for presentation	Exposure (h)	Mucous membranes	CRT	Heart Rate	Respiratory Rate	Temperature
A	2,25	F	lily	12	pink	<2	240	30	39,1
B	3,2	M	lily	12	pink	<2	152	40	38,4
C	0,4	M	lily	2	pink	<2	200	40	39,1
D	0,5	M	lily	2	pink	<2	148	28	38,8
E	7,9	M	lily	5,5	pale pink	<2	170	-	37,7
F	3,2	M	lily	1,5	-	-	-	-	-
G	7,2	F	lily	1,5	-	-	-	-	-
H	0,6	F	lily	-	-	-	-	-	-
I	6	F	lily	8	pink	<2	210	40	38,6
J	12	F	lily	2	pink	<2	-	-	-
K	5,5	F	lily	-	-	-	200	48	38,4

	NIBP	Glucose (mmol/l)	Lactate	PCV (%)	Albumin (g/l)	Platelet count (K/µL)	Mentation Score	Apple Fast Score
A	150	6,1	1,6	46	36	270	0	14
B	158	5,8	2,1	38	38	216	0	13
C	104	-	2,8	-	-	-	0	-
D	-	-	-	-	-	-	0	-
E	120	7,3	1,5	26	-	-	0	13
F	-	5,4	1,3	40	-	-	0	-
G	-	8,7	5,6	42	38	183	0	-
H	-	4,2	-	32	38	617	0	-
I	-	-	-	46	-	-	0	-
J	-	-	-	46	-	-	0	-
K	-	-	-	-	-	-	0	-

	Cr t=0h	Cr t=24h	Cr t=48h	Cr t=72 h	Above RN 0h	Above RN 24h	Above RN 48h	Above RN 72h	Cr range	IRIS criteria	
A	121	133	129	120	N	N	N	N	NAKI	NAKI	D
B	166	124	135	123	N	N	N	N	NAKI	NAKI	D
C	66	69	60	68	N	N	N	N	NAKI	NAKI	D
D	77	70	65	96	N	N	N	N	NAKI	NAKI	D
E	149	175	194	246	N	N	N	Y	AKI	AKI	D
F	189	164	147	166	N	N	N	N	NAKI	NAKI	D
G	168	161	159	155	N	N	N	N	NAKI	NAKI	D
H	133	122	120	110	N	N	N	N	NAKI	NAKI	D
I	187	129	139	166	N	N	N	N	NAKI	NAKI	D
J	203	121	177	172	N	N	N	N	NAKI	NAKI	D
K	204	197	216	240	N	N	Y	Y	AKI	AKI	D

	Weight 0h (kg)	Weight 12h	Weight 24h	Weight 36h	Weight 48h	Weight 60h	Weight 72h	Fluid Rate 0h	FR 24h	FR 48h	FR 72h
A	3,7	3,7	3,6	3,6	3,6	3,5	3,7	4	4	4	4
B	7,4	7,5	7,5	7,3	7,4	7,4	7,4	4	4	4	4
C	2	2	2,01	2,1	2,1	2,1	-	4	4	4	4
D	3,4	-	3,4	-	3,2	3,3	3,4	4	4	4	4
E	4,5	4,4	4,4	4,4	4,5	4,5	4,4	4	4	4	4
F	5,7	5,8	5,7	5,5	5,6	-	5,5	4	4	4	4
G	6	-	6,1	6,2	6	5,9	6	4	4	4	4
H	2,4	2,4	2,3	2,3	2,4	2,3	2,3	4	4	4	4
I	4,2	4,4	-	4,3	-	4,2	-	4	4	4	4
J	3,5	3,8	3,6	3,4	3,5	3,5	-	6	2	2	2
K	4,2	4,3	4,3	-	-	4,3	4,4	6	6	-	4

Differences between each day weights (in percentages)

A	0 %	-3 %	0 %	0 %	-3 %	5 %
B	1 %	0 %	-3 %	1 %	0 %	0 %
C	0 %	0 %	4 %	0 %	0 %	-
D	-	-	-	-	3 %	3 %
E	-2 %	0 %	0 %	2 %	0 %	-2 %
F	2 %	-2 %	-4 %	2 %	-	-
G	-	-	2 %	-3 %	-2 %	2 %
H	0 %	-4 %	0 %	4 %	-4 %	0 %
I	5 %	-	-	-	-	-
J	8 %	-6 %	-6 %	3 %	0 %	-
K	2 %	0 %	-	-	-	2 %

APPENDIX III - DATA COLLECTED IN THE CANINE GROUP

	Age (years)	Sex	Reason for presentation	Emesis	Exposure (h)	Mucous membranes	CRT	Heart Rate	Respiratory Rate	Temperature
A	1,3	M	raisins	Y	0,75	pink	<2	-	-	-
B	1,75	F	raisins	-	5	pink	<2	120	32	39,1
C	5	F	raisins	-	5	pink	<2	80	-	38
D	1,25	M	raisins	N	12	pink	<2	120	40	39
E	4,5	F	raisins	Y	4,5	pink	<2	136	44	39
F	1	F	raisins	N	9	pink	-	-	-	-
G	1,8	M	raisins	Y	0,5	pink	<2	120	28	38

	NIBP	Glucose (mmol/l)	Lactate	PCV (%)	Albumin (g/l)	Platelet count (K/µL)	Mentation Score	Apple Fast Score
A	-	4,9	-	44	37	215	0	-
B	-	5,5	-	48	41	268	0	-
C	-	4,8	-	42	42	183	0	-
D	145	5	1,1	57	42	203	0	13
E	116	6,1	3,2	49	40	293	0	15
F	-	-	-	-	-	-	0	-
G	-	-	-	58	-	415	0	-

	Cr t =0h	Cr t=24h	Cr t=48h	Cr t=72 h	Above RN 0h	Above RN 24h	Above RN 48h	Above RN 72h	Cr range	IRIS criteria	
A	92	109	83	95	N	N	N	N	NAKI	NAKI	D
B	119	100	97	105	N	N	N	N	NAKI	NAKI	D
C	106	91	101	106	N	N	N	N	NAKI	NAKI	D
D	117	94	98	79	N	N	N	N	NAKI	NAKI	D
E	108	-	116	111	N	N	N	N	NAKI	NAKI	D
F	120	110	107	102	N	N	N	N	NAKI	NAKI	D
G	91	86	83	89	N	N	N	N	NAKI	NAKI	D

	Weight 0h (kg)	Weight 12h	Weight 24h	Weight 36h	Weight 48h	Weight 60h	Weight 72h	Fluid Rate 0h	FR 24h	FR 48h	FR 72h
A	22	22,4	22,2	22,3	22,2	21,1	21,9	4	4	4	4
B	27,2	27,2	27,1	26	25,9	26	26	4	4	4	4
C	26,2	26,2	26,2	26,2	26	25,8	25,9	4	4	4	4
D	19	19,7	-	19,8	19,4	19,6	19,3	4	4	4	4
E	18,2	17,5	17,6	17,5	17,7	17,7	17,8	4	4	4	4
F	-	-	18,4	-	18	-	18,1	4	4	4	2
G	9	9	9,1	9,2	9,2	9,3	9,4	4	4	4	4

Differences between each day weights (in percentages)

A	2 %	-1 %	0 %	0 %	-5 %	4 %
B	0 %	0 %	-4 %	0 %	0 %	0 %
C	0 %	0 %	0 %	-1 %	-1 %	0 %
D	4 %	-	-	-2 %	1 %	-2 %
E	-4 %	1 %	-1 %	1 %	0 %	1 %
F	-	-	-	-	-	-
G	0 %	1 %	1 %	0 %	-1 %	1 %

APPENDIX IV – APPLE full & fast SCORE FOR DOGS

APPLE$_{full}$ Score US units

								creatinine (mg/dL) 0-0.62	*1* 0.63-1.35			*8* 1.36-2.26			*9* >2.26				
				9 <5.1				wbc (x10^9/l) 5.1-8.5	*2* 8.6-18			*3* >18							
6 <2.6			*7* 2.6-3.0			*9* 3.1-3.2		albumin (g/dL) 3.3-3.5	*2* >3.5										
10 <90			*4* 90-94				*1* 95-97	SpO_2 (%) 98-100											
								total bilirubin (mg/dL) 0-0.23	*6* 0.24-0.46			*4* 0.47-0.93			*3* >0.93				
								mentation score 0	*5* 1			*7* 2			*8* 3			*13* 4	
								respiratory rate (bpm) <25	*3* 25-36			*5* 37-48			*6* 49-60			*5* >60	
								age (years) 0-2	*6* 3-5			*8* 6-8			*7* >8				
			3 2			*4* 1		fluid score 0											
								lactate (mg/dL) <18.0	*2* 18.0-71.2			*3* 71.3-90.1			*6* >90.1				

Footnote: See Table 3 for calculation of 'fluid score' and 'mentation score'. A value of zero is ascribed for each parameter in the central zone. 'Mentation score' is collected at admission, for all others utilise the most abnormal value identified over the 24 hour period following admission. If history and physical exam fail to prompt assessment of $Sp0_2$ or fluid score, assign zero.

APPLE$_{fast}$ Score US units

7 <84			*8* 84-102			*9* 103-164		*10* 165-273	**glucose (mg/dL)** >273										
8 <2.6			*7* 2.6-3.0			*6* 3.1-3.2			**albumin (g/dL)** 3.3-3.5	*2* >3.5									
									lactate (mg/dL) <18.0	*4* 18.0-72.1			*8* 72.2-90.1			*12* >90.1			
		5 <151			*6* 151-200			*3* 201-260	**platelet count (x10^9/L)** 261-420,000	*1* >420									
									mentation score 0	*4* 1		*6* 2			*7* 3			*14* 4	

Footnote: See Table 3 for calculation of 'mentation score'. A value of zero is ascribed for each parameter in the central zone. 'Mentation score' is collected at admission, for all others utilise the most abnormal value identified over the 24 hour period following admission.

APPENDIX V - APPLE full & fast SCORE FOR CATS

US Unit Models

				Mentation score 0	*4* 1	*7* 2	*8* 3	*9* 4					
	6 <36.1	*4* 36.1-37.0	*3* 37.1-38.5	**Temperature (C)** 38.6-39.4	*1* >39.4								
9 <61		*4* 61-100		**MAP (mmHg)** 101-140	*1* >140								
				lactate (mg/dL) 0-17.1	*5* 17.2-36.0	*6* 36.1-63.1	*9* >63.1						
				PCV(%) <11	*11* 11-20	*16* 21-30	*14* 31-40	*13* 41-45	*17* >45				
11 <14.9		*7* 14.9-21.3		**Urea (mg/dL)** 21.4-24.9	*12* 25.0-32.5	*7* 32.6-69.8	*6* >69.8						
12 <111		*9* 111-115	*11* 116-118	**Chloride (mEq/L)** 119-122	*11* 123-125	*7* >125							
				Body cavity fluid score 0	*3* 1	*6* 2							

Fig A1. Feline Acute Patient Physiologic and Laboratory Evaluation ($APPLE_{full}$) score conventional (US) units: Calculated by summing the value in the upper left corner of the appropriate cell for each of the 8 parameters listed, with a maximum potential score of 80. The central cell in each figure represents the range of values for the variable for which 0 points would be assigned. The cells to either side show the appropriate score for the corresponding range of the variable. The final score for the patient is achieved by summing the scores for each variable. See Table 3 for calculation of "fluid score" and "mentation score." "Mentation score" is collected at admission, for all others utilize the most abnormal value identified over the 24-hour period following admission. If history and physical exam fail to prompt assessment of fluid score, assign zero. Mean arterial pressure (MAP) reflects the value recorded for MAP if direct or indirect manometry is used, or the pressure corresponding to 1st return of audible signal on cuff deflation if Doppler is used.

				Mentation score 0	*5* 1	*6* 2	*7* 3	*10* 4				
6 <35.6	*5* 35.6-36.0	*4* 36.1-37.0	*3* 37.1-38.5	**Temperature (C)** 38.6-39.5	*1* >39.5							
11 <61		*7* 61-100		**MAP (mmHg)** 101-140	*1* >140							
				lactate (mg/dL) 0-17.1	*6* 17.2-36.0	*9* 36.1-63.1	*10* >63.1					
				PCV(%) <16	*12* 16-25	*10* 26-35	*9* 36-45	*13* >45				

Fig A2. Feline Acute Patient Physiologic and Laboratory Evaluation ($APPLE_{fast}$) score conventional (US) units: Calculated by summing the value in the upper left corner for each of the 5 parameters listed, with a maximum potential score of 50. The central cell in each figure represents the range of values for the variable for which 0 points would be assigned. The cells to either side show the appropriate score for the corresponding range of the variable. The final score for the patient is achieved by summing the scores for each variable. "Mentation score" is collected at admission (see Table 3), for all others utilize the most abnormal value identified over the 24-hour period following admission. Mean arterial pressure (MAP) reflects the value recorded for MAP if direct or indirect manometry is used, or the pressure corresponding to 1st return of audible signal on cuff deflation if Doppler is used.